	FOOD SAFETY LAB / MILK QUALITY IMPROVEMENT PROGRAM *Standard Operating Procedure*	

Title: EddyJet2 Spiral Plater and SphereFlash Colony Counter SOP

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EddyJet2 Spiral Plater and SphereFlash Colony Counter SOP

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SECTION 1   INTRODUCTION

1.1   Purpose

The purpose of this document is to set forth standard guidelines for operation of the EddyJet 2 Spiral Plater and accompanying SphereFlash Colony Counter device.

1.2   Scope

This SOP applies to the Food Safety Lab and all associated personnel who will be using these devices.

1.3   Safety

These devices are located in a BSL2 lab and will have direct exposure to human pathogens during regular use. Take all necessary precautions while using these devices to protect against potential exposure to these organisms, even when not working with them directly. Both of the devices have moving parts, and precautions should be taken not to obstruct or otherwise interfere with these moving elements during use to avoid harm to the user or the device.
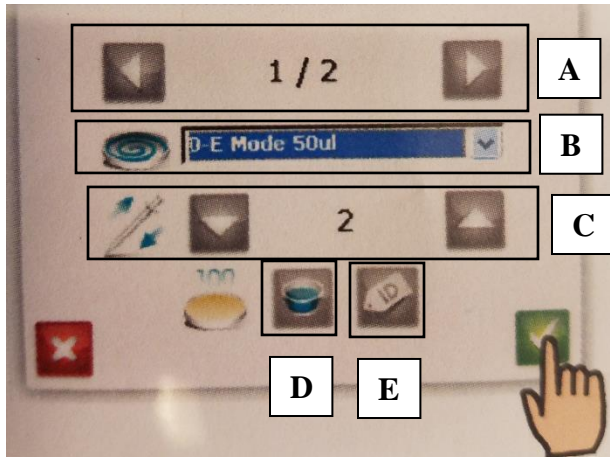
SECTION 3   PROCEDURES

3.1. EddyJet 2 Spiral Plater Operation

For detailed information on the operation of the EddyJet 2, refer to the guide manual located with the device.

3.1.1 Starting the EddyJet2

(1) Turn on the device with the power switch on the rear-left side of the device.
(2) Remove the dust shield and set aside

3.1.2 Sample Setup

(1) From the home screen, navigate to the plating setup screen by selecting the blue tip icon.
(2) Select the number of replicates for the sample by using the arrow buttons then press the green check to confirm
(3) Select the plating mode (B) from the dropdown menu.
    a. E-mode 50ul is the standard plating mode. For more detailed information concerning the volume settings for the other plating modes, see Appendix 1
(4) The default purge cycle (C) is 2 and should not be changed unless excessive bubbles are seen in the tip during plating.
(5) Select the feeding mode (D) as either sample cup or tube. Typically samples will be in tubes, so the tube setting should be selected.
(6) ID (E) should be left inactive unless barcoding of samples is used.
(7) Use the left and right arrows (A) to select each replicate and adjust the settings as previously described for each replicate of the sample as needed. Press the check button when settings are complete.

3.1.3 Plating a Sample

(1) Place the next agar plate into the plate carriage, ensuring it is fully secure and flat on the carriage, then remove the lid from the plate
(2) If using a sample cup, place the filled sample cup into the trough
(3) Press the green play button from the home screen to start the plating process.
(4) If prompted to remove or insert new syringe tips at any point during this process, see section 3.1.4
(5) If using a tube:
    a. The device will load a syringe and wait for user input
    b. Vortex sample tube, then bring it up to the syringe and immerse syringe into sample.
    c. Press the green check button to take up sample into the syringe
        i. Ensure that the syringe does not take up air as the volume in the tube decreases
    d. Once the volume has been taken up, press the green check button to plate the sample.
    e. After the sample is plated, the device will return to the sample uptake position and retain the same syringe.
    f. Replace the lid on the plate and remove it from the device, then load the next plate.
    g. Repeat step 5 for all replicates of the sample
(6) If using a sample cup:
    a. The device will load a syringe and take up sample, then deposit it on the plate according to the plating mode selected
    b. The device will then return to the sample uptake position
    c. Replace the lid on the plate and remove it from the device, then load the next plate.
    d. Press the green check button to plate the next replicate
    e. Repeat step 6 for all replicates of the sample
(7) When the last sample replicate has been plated, the syringe tip will be automatically discarded and the device will return to the home screen.
(8) If a sample cup was used, remove it from the device and discard appropriately
(9) Repeat steps 1-8 for all samples.
(10) If any issues arise during the plating process, use the red X button to cancel the plating

3.1.4 Changing the syringe tip box

DO NOT remove or insert a new box unless prompted to do so by the device

(1) When prompted to remove the tip box with this symbol, remove the empty box from the device.
(2) When prompted to load a new tip box with this symbol take a new box from the tip box case located next to the device

(3) GENTLY insert the tabbed side of the tip box into the device, ensuring that the words on the side of the box are right side up (the words should not be upside-down)
(4) The machine should recognize the insertion of the box and load a new tip.
    a. If this does not occur, first make sure the machine has prompted for a new box then ensure the box has been inserted the proper direction (the tab side goes in and words should be right side up)
    b. If the machine has a problem loading a new tip (stuck in the loading area, loud motor noise) press the red X or turn off/on the machine and retry sample.

3.1.5 Shutting down the device

(1) Discard the remaining sample cup if necessary.
(2) Replace the dust shield on the device
(3) Shut down the device with the power switch on the back.
(4) Remove the tip waste bin and discard the tips in the **BSL2** waste
    a. This should be done even if only working with BSL1 organisms, as the waste container may contain organisms from previous platings
(5) Spray the tip waste bin with 70% ethanol and return it to the device
(6) Clean area around plater with 70% ethanol

3.2 SphereFlash Colony Counter Operation

For detailed information on the operation of the SphereFlash, refer to the guide manual located with the device.

The SphereFlash has a moving sphere cover which opens and closes regularly during use. Do not put anything under the cover while it is open other than to insert and remove plates. Do not attempt to restrict the movement of the sphere cover while opening or closing, as this could damage the device and/or harm the user.

3.2.1 Starting the SphereFlash

(1) Turn on the computer and the monitor and log in using the provided credentials
    a. Do not turn on the SphereFlash until you have finished logging in.
(2) Turn on the device by pressing the power button on the front
    a. A red light will turn on and will begin to flash after ~40 seconds
    b. The sphere cover will rise, indicating the device is ready
(3) Wipe down the surface of the glass platform to remove any moisture or debris.
(4) Open the SphereFlash software and login using the provided credentials

3.2.2 Imaging Setup

(1) Select "Colony Count" from the side menu
(2) Choose the proper results group from the dropdown menu or create a new results group database if necessary. The default Count Results group can be used, but it will not be as easy to find old results when sorting by result group
(3) Load a plate into the device, ensuring it is secure and flush against the glass platform
(4) Input the Plate ID and any comments necessary
(5) Select the appropriate imaging method to be used. See sections 3.2.4 & 3.2.5 for information on choosing and creating image methods.
(6) The "Inoc mode" must match the plating method used on the EddyJet2 or while spread plating.
    a. This device can only be used to count colonies on spiral plates from the EddyJet2 or manually spread plates.
(7) If spread plates are used, the dilution factor and plating volume must be input.
    a. The dilution input value is the ratio of the original sample to the current dilution, meaning a 2 log dilution ("-2") needs an input of 100 for the dilution, as this would be a 1:100 dilution. (This value is a "1" followed by the same number of zeros as the log dilution, ex: a 5 log dilution or "-5" is 100000 dilution input)
    b. Volume is in mL (100µl plating = 0.1 mL input)

3.2.3 Colony Counts

(1) Ensure that the SphereFlash platform is unobstructed, as the sphere cover will close during imaging
(2) Press the "Play" button in the program window or the physical button on the device when the plate is loaded with the lid off and the image settings are properly setup
(3) The sphere cover will close and an image will be taken.
(4) View the resulting image and colony count to ensure accuracy.
(5) Identified colonies will each be outlined in the image, and each counted colony will have an X in the center.
    a. If colonies have not been counted, or if too many non-colonies (such as debris in or on the agar) have been counted, first adjust the "Thr. to back" slider so that all the outlined elements in the image are the colonies to be counted.
    b. Then adjust the "Absolute min. diameter" slider so that every colony has been marked by an X, and non-colonies are not marked with an X.
    c. If the count is not accurate and cannot be fixed using the above steps, press the "X" discard button and refer to section 3.2.5
(6) If replicate plates are to be counted, load the next plate and press the "New Image" button again. This will save the previous count result and image the newly loaded plate, adding a sequential number to the plate ID.
(7) When all replicates have been imaged and a new sample plate will be loaded, press the "Save Results" button.
(8) Repeat steps 1-7 for all plates to be counted

(9) When the last plate has been counted, highlight the data to be saved in the results table
(10) Right click the highlighted data and select "Export Data"
    a. Images will be saved by the SphereFlash software for future reference, and should not be exported unless needed, as they will use a large amount of server storage
(11) Save data to the proper location on the lab server.

3.2.4 Choosing a Method

(1) The SphereFlash has many different pre-defined methods for counting colonies on a plate
    a. Default methods have descriptions of the lighting that will be used and colony type on the plate
    b. Choose the method that best suits your organisms. This can be changed for every plate
(2) If none of the default methods are appropriate or accurate for counting your plates, refer to section 3.2.5

3.2.5 Creating a new Method

(1) If the default methods are not sufficient to count your plates, you may need to modify an existing method or create a new method.
(2) Select the method that is closest to the desired settings and press the "Edit Method" button (+ symbol)
(3) Modify the parameters of the method to accurately count the plates needed. Information on the details of these parameters can be found in the SphereFlash Software User Guide located with the device.
    a. Consult with Jordan Skeens, Sam Reichler, or other senior lab members and technicians for assistance in the setup of these methods.
(4) Be sure to utilize multiple test plates while creating a new Method to ensure the changed parameters are having the desired results and producing accurate counts.
(5) When the test plates are all providing accurate counts, save the new Method.
    a. Be sure to rename the method signifying the changed parameters or specific use the method is applicable to.

3.2.6 Turning off the SphereFlash

(1) Wipe down the surface of the glass platform to remove any moisture or debris.
(2) Press the power button on the front of the device
(3) The sphere cover will close and the red light will flash for ~5-10 seconds
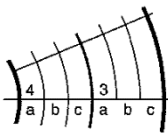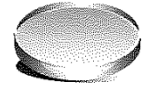(4) Shut down the computer and turn off the monitor

APPENDIX 1:

Volume (µl) Table: 100 mm Petri Dishes

EDDY JET 2

90 - 100 mm

Counting Grid

	3c	3c+3b	3c+3b+3a	3+4c	3+4c+4b	total
E-Mode 50 µl	2,56	6,44	12,08	20,36	32,32	50
C-Mode 40 µl	1,52	3,96	7,68	13,36	22	37,3
C-Mode 100 µl	4,56	11,52	21,52	35,92	56,8	92,2
D-Mode 49,2 µl	2,16	5,48	10,56	18,28	30	49,2
D-Mode 50 µl	3	7	13,2	22	34	50
Exp. 3000 Mode 50µl	1,16	4,72	10,08	18,28	30,12	50
Log Mode 50 µl	2,16	5,48	10,56	18,28	30	49,2
Log Mode 100 µl	4,32	10,8	21,12	36,4	60	98,4
Log Mode 200 µl	8,64	21,6	42,24	72,8	120	196,8
Linear Mode 18µl	3	6	9	12	15	18
Linear Mode 50µl	8,33	16,67	25	33,33	41,67	50
Linear Mode 100µl	16,67	33,34	50	66,67	83,34	100
Linear Mode 200µl	33,33	66,67	100	133,33	166,67	200
Slow 3000 Mode 50µl	1,16	4,72	10,08	18,28	30,12	50
Prop. 3000 Mode 20µl	3,33	6,66	10	13,33	16,67	20
Lawn 3000 Mode 20µl	4,38	8,23	11,56	14,37	16,66	20
Unif. 3000 Mode 20µl	4,73	8,91	12,52	15,58	18,09	20,04
C-Mode Fast 40 µl	1,52	3,96	7,68	13,36	22	37,3
Log Mode 300 µl	12,96	32,4	63,36	109,2	180	295,2
Log Mode 400 µl	17,28	43,2	84,48	145,6	240	393,6
Linear Mode 300µl	50	100	150	200	250	300
Linear Mode400µl	66,67	133,33	200	266,67	333,33	400

SECTION 4   TROUBLESHOOTING

Refer to the user guides located with the devices to troubleshoot minor issues.

All issues with either the EddyJet2 or the SphereFlash should be reported to Sam Reichler or Jordan Skeens

Known EddyJet issues:
Syringe tip does not fall to plate during spiral process – Report to Jordan or Sam and refer to the following video: https://www.youtube.com/watch?v=FoYKIutL6sg&t

No lab users should attempt technical maintenance or modifications of the devices without discussing with NeuTec support personnel. See below:

NeuTec support: 888-810-5179 Opt. 3

EddyJet ID: 10003700 / 579

SphereFlash ID: 90007000 / 0064

SECTION 5   REFERENCES

All protocols in this document were derived from the manufacturer's user guides provided with the devices. These user guides can be used to aid in more advanced operation of the devices as well as troubleshoot any issues.

SECTION 6   METHOD VERSION & CHANGES

VERSION	DATE	EDITOR	COMMENTS
Version 1	08/21/2019	Jordan Skeens	Original SOP
Version 2	04/21/2020	Jordan Skeens	Updated to 04/2020 SOP template, Title page: updated the SOP# to 5.3.5