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Microbiology Laboratory
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Standard Operating Procedure for
Use of the PetriSwiss PS200 Instruments

SOP Number: EQ-12-01

Date Revised: 09-28-21

SOP Number	EQ-13-01
Title	Use of the PetriSwiss PS200 Instrument
Revisions Made	• Minor editorial changes for clarification purposes. • Section 12 was expanded to include loading the rack with petri dishes, installing the filling tube assembly, rinsing the agar containers, cleaning of the tube assembly, and the instrument.

SOP Number	EQ-13-01
Title	Use of the PetriSwiss PS200 Instrument
Scope	This SOP describes the use of the semi-automated PetriSwiss PS200 to dispense agar-based media into Petri dishes
Application	This SOP is used to dispense agar-based media with the PS200 instrument.

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1. Definitions	1. Screen = one of the several visual displays which may appear on the front of the PS200. 2. Key = a display section on the screen. The most used keys are blue squares with green symbols. 3. Button = a push button on the front of the PS200. There are two buttons: one is labeled START/BRAKE and the other is labeled PUMP/MANU [manual]. 4. Filling chamber = the area under the hinged cover. 5. Filling tubing assembly (delivery tube) = consists of sections of plastic tubing with a metal nozzle at one end, one open end which goes into the agar solution, and a middle section of dual tubing for the two channels of the pump. 6. Dish and plate = refer to the plastic Petri dish. 7. Rack = a removable tower which holds the plastic Petri dishes. 8. The "carousel" or "carousel disk" = the carousel which holds the Petri dish racks. 9. The "dish separator" = the small carousel in the filling chamber.
2. Health and Safety	Follow procedures specified in SOP MB-01, Laboratory Biosafety.
3. Personnel Qualifications and Training	Refer to SOP ADM-04, OPP Microbiology Laboratory Training.
4. Instrument Calibration	1. The delivery volume may change after many autoclaving cycles because of loss of tube flexibility, etc. 2. The volume may be checked and adjusted. 3. With the double tubing section of the filling tube assembly installed in the pump, put the nozzle end and the filling inlet end into a container of water (e.g., 400 mL beaker) 4. Turn on power to get PS200/PS400 screen. 5. Press green check-mark key (✔) to get the HOME screen. 6. While on the HOME screen and with the filling tube assembly installed as described above, press the PUMP/MENU button (to the right of the display screen) to fill the filling tube assembly with water. 7. On the HOME screen, press the PUMP key to get the PUMP screen.

	8. On the PUMP screen, press the key with the green arrow, repeatedly if necessary, to make sure the filling tube assembly is completely filled with water. 9. Then, press the CAL key to get the PUMP CALIBRATION screen. 10. On the PUMP CALIBRATION screen, press the SPEED key until the speed selection is green for the speed parameter of the method. 11. Place the nozzle end of the filling tub to deliver into a graduated cylinder (e.g., 50 mL). 12. Then, press the key with the green arrow. The instrument will pump the volume set in the method and display the PUMP CALIBRATION screen with a "Volume Correction" option. **Note the volume delivered.** 13. If the delivered volume is correct, press the green check mark. Then, on the PUMP screen, press the HOME key (key with house) at the lower right. The calibration remains the same. 14. If the delivered volume is not correct, press the up or down arrow key to enter the delivered volume on the "Volume Correction" screen. Press the green check mark. Then, on the PUMP page, press the HOME key (key with house. The revised calibration is set. 15. On the PUMP screen the options include: a. The HOME key which causes the volume correction to be saved. b. The key with a green arrow which causes the corrected volume to be dispensed so that the delivered volume may be checked. Then, the HOME key may be pressed to save the volume correction and return to the HOME screen, c. CAL key which restarts the volume correction process. Note that the previously entered volume correction is **not** saved.
5. Sample Handling and Storage	1. Dispense agar into plates as specified by the individual preparation sheets. 2. The temperature of the agar to be dispensed needs to be higher than for manual pouring of plates because the agar will cool when travelling through the filling tube and nozzle. Cooling will be greater between rack changes and agar solution container changes when the agar is not flowing.
6. Quality Control	1. The delivered volume should be calibrated when a new filling tube is used and after a tube has been sterilized several times (approximately15-20 times). 2. The delivered volume should be checked by the user quarterly.

	3. Calibration is specific to the type/brand of Petri dish, conduct calibrations accordingly.
7. Interferences	1. Broken plates and plates loaded incorrectly. 2. A change in silicone tube flexibility.
8. Non-conforming Data	1. Management of non-conforming data will be consistent with SOP ADM-07, Non-Conformance Reports.
9. Data Management	1. Data will be archived consistent with SOP ADM-03, Records and Archives.
10. Cautions	1. Do not put hands or fingers near the carousel, racks, or filling chamber when an operation is being performed or initiated. 2. Do not use harsh chemicals in cleaning. Agar spills may be removed with a water-moistened towel or tissue. Disinfection may be done with a tissue, towel or swab that is moistened with 70% ethanol in water. 3. The agar will cool while travelling through the filling tubing. If the agar is too cool the filling tube will clog. 4. Do not use PetriSwiss instrument for dispensing agar media that requires addition of enrichments, a heat-sensitive step, e.g., 7H11 agar.
11. Special Apparatus and Materials	1. Water bath to hold agar at 57±2º C. Monitor temperature of water bath by using a thermometer inside a flask of water kept inside the water bath. 2. Use ring weights to stabilize the agar containers in the water bath. 3. Polystyrene Petri dishes, size 100 mm × 20 mm, slippable, Excel Scientific D-905, Sigma-Aldrich number P5606-400, or other comparable, slippable Petri dishes.
12. Procedure and Analysis	1. The instrument should be operated according to manufacturer's instructions. 2. Instrument parameters for filling plates are contained in a software program. Software programs may be created and edited (as noted below) by users. Parameters in a program include the following: a. Filling volume: set according to media preparation sheet; typically, 25, 30 or 50 mL b. Dish height: set for 21.3 mm for polystyrene Petri dishes, size 100 mm × 20 mm, slippable, Excel Scientific D-905, Sigma-Aldrich number P5606-400. c. Customary settings for the other parameters are:

	i. Pause Time: 0.0 seconds ii. Filling speed: Depends on tube size and calibration; typical values are 663 mL/minute and 708 mL/minute. iii. Cooling Tower: not applicable (N/A) iv. UV Light Anti Drop: On v. Ink-Jet: N/A vi. Speed/Mix: Fast 3. The tasks of sections 12.1 through 12.3 and the first three steps of section 12.4 (up until the UV lamp comes on) may be performed while the agar is being autoclaved and placed in the water bath.
12.1 Disinfecting the filling area	a. Before using the PS200 for filling plates, disinfect (using a tissue, towel or swab moistened with 70% ethanol) the dish separator, the agar tray (the platform on which the dish separator rests), and the brackets (inside and outside) that hold the filling nozzle in place. Include the areas on which the pistons (dish lifters) rest. b. Use the Manual keys to manipulate the unit as desired. The Manual keys are available from the HOME screen (see steps 3 and 4 under "Instrument Calibration" above) by pressing the MANUAL key. c. Close the hinged cover over the filling chamber.
12.2 Loading the racks with petri dishes	a. Fill the racks with petri dishes (plates) as needed. A 3-L batch of agar will fill 100-120 plates with 25 mL/plate. Sixteen plates may be placed in each of 7 racks and several plates may be place in the 8th rack. b. Load racks of plates on their carousel, using the left and right arrow keys at the center of the screen to move the carousel for convenience in loading. (The first rack should be empty to receive the plates to be filled.) c. Place the empty column rack in position A (labelled as "Pos. A" on the instrument) using the same keys. d. Place an empty rack in "Pos. A" (as labelled on the instrument) on the carousel. This is the first of the racks to be used. e. Place the filled racks on the carousel following the empty rack.
12.3 Installing the filling tube assembly	a. Move the lever above the pump to its right-most position.

	b. Install the double section of the (previously autoclaved) filling tubing assembly into the two channels of the pump, placing the nozzle end of the assembly on the left. c. Remove the foil from the nozzle end of the assembly and install the nozzle into its brackets. (There are two brackets for the filling tube nozzle: one is on the perimeter of the hinged cover over the pump and the second is inside the filling chamber between the first bracket and the plate separator.) The nozzle gives a click sound when it is properly inserted in the second bracket.
12.4 Performing the filling procedure	a. Turn on power to get PS200/PS400 screen. b. Press green check-mark key to get the HOME screen. c. On the HOME screen, press FILL key. Unit moves to position A and displays FILL screen. d. On the FILL screen, press the green arrow key to begin operation with the program shown (or press the LIST key to select a program, or press the EDIT key to make a change in the program or to check a program parameter value (e.g., fill volume, etc.). The UV lamp comes on. Get Set Dish Count screen. e. On the Set Dish Count screen, input number of dishes to fill (or a number that is greater than the number anticipated). Press the green arrow check-mark key to get the PREPARE FILLING screen. (If a WARNING screen with "RESET DISH COUNT?" message comes up, press the green check-mark key.) f. Transfer the agar from the autoclave to the water bath, held at 57±2ºC, if this has not been done earlier. Use weights to stabilize the agar containers in the water bath. g. Press the green arrow at the **far right** of the PREPARE FILLING screen to get the Process Status screen. h. As the process begins, press the START/BRAKE button. The process begins with moving of racks, checking the plate separator to make sure there are no plates already there, and lowering the first plate to be filled. The START/BRAKE key must be pushed before the first plate is in position to be filled. When this process is completed, the PAUSE screen appears. i. While on the PAUSE screen, remove the foil from the other end of the assembly and put the tubing in the agar solution (which is in a water bath or has just been moved out of the water bath).

	j. Move the lever above the pump to its left-most position. k. Press the PUMP/MANU button to fill the filling tubing assembly with agar up to the nozzle. l. Press the green arrow key to get the FILLING PROCESS screen. The filling process continues. Note: if the filling is to be from more than one container of agar, the first container may be moved out of the water bath after the filling operation begins to prevent contamination of the bath when the filling tube is moved to the next container. m. When an agar container has insufficient agar to fill the next plate, press the START/BRAKE button to pause the filling process. The display will show the PAUSE screen. n. Remove the next agar container from the water bath and place the filling tube in it. o. Press the green arrow key to continue the filling operation on the FILLING PROCESS screen. p. Return the emptied container to the water bath to keep the remaining agar from solidifying before the container is rinsed. Weights should remain on the container to stabilize it in the bath. q. When the number of plates filled reaches the dish count value entered earlier, the filling operation stops but all the plates in the current rack will be transferred. r. If the agar supply is exhausted, press the orange key at the **far bottom** right of the FILLING PROCESS screen. The Process Status icon will turn orange. The unit will stop filling plates, but it will empty the current rack before stopping.
12.5 Cleaning the filling tubing assembly	a. When the unit has stopped filling plates, transfer the nozzle to a container (e.g., the last agar container just used) and the tubing end to a container of (preferably hot) distilled water. This may be a 500-mL container from the water bath. b. When the unit has emptied the last rack and returned to the HOME screen, press and hold the PUMP button to rinse the filling tubing. c. Remove the filling tubing assembly from the pumping mechanism, rinse the ends with hot tap water followed by distilled water. d. Allow the tubing assembly to drain and set it aside for autoclaving.

12.6 Rinsing the agar containers	a. Remove used agar containers from water bath, rinse with hot tap water, and put in dishwasher. If containers set for too long, the agar will be difficult to rinse away.
12.7 Cleaning the filling area of PS200	a. Raise the hinged cover. b. Remove the plate separator and the agar tray (the platform on which the plate separator rests). c. Clean away any spilled agar with a water-moistened towel. d. Clean and disinfect with a tissue, towel or swab moistened with 70% ethanol, the plate separator, the agar tray, the pistons (dish lifters) and the brackets that hold the filling nozzle in plate. Use the Manual keys to manipulate the unit as desire. e. Close the hinged cover. f. Turn off power switch of the PS200. g. Allow the dishes to remain in their racks until the agar has solidified.
12.8 Sterilization of the filling tubing assembly	a. Dry the filling tubing assembly before autoclaving. (Room air may be drawn through the assembly with a vacuum source or compressed air may be blown through the tubing assembly to dry it.) b. Wrap aluminum foil around the nozzle and at least six inches of the tubing where the nozzle is attached. c. Wrap aluminum foil around at least 14 inches of the other end of the tubing assembly. d. Seal the assembly in an autoclave pouch. e. Autoclave the assembly at 121° C for either 20 or 25 minutes on a gravity cycle. Time is selected based on any other items that may be conveniently autoclaved at the same time.
13. Data Analysis/ Calculations	None
14. Forms and Data Sheets	Test sheets are stored separately from the SOP under the following file name: PetriSwiss Tubing Calibration Log EQ-13-01_F1.docx
15. References	PetriSwiss PS-200 User Manual 1.0-E, Version 2.0