

SOP: Aseptic Processing: Media Transfer Using Tube Welding

Approvals:

Preparer: Bryn Mallon

Date: 12MAY2023

Reviewer: Hetal Doshi

Date: 21JUNE2023

Reviewer: Emily Dentinger

Date: 18JULY2023

Reviewer: Dr. Maggie Bryans

Date: 23JULY2023

1. Purpose:

- 1.1. To transfer cell culture media from a single use sterile media bottle to a single use cell culture bag using a Masterflex 3-easy pump and sterile tube welder.

2. Scope:

- 2.1. This SOP covers the use of the MasterFlex 3- easy pump and the sterile tube welder to aseptically transfer cell culture media from a sterile cell culture media bottle to sterile cell culture bag.

3. Summary of Method:

- 3.1. Set up pump with correct tubing.
- 3.2. Place tubing in correct orientation in the welder and weld the tubes together.
- 3.3. Turn on pump and allow for flow into cell bag.

4. References:

- 4.1. MasterFlex L/S digital pump drive manual
- 4.2. Gowning SOP: QCM 4
- 4.3. TerumoBCT SCD 11B sterile tube welder operating instructions
- 4.4. Calibration of MasterFlex L/S pump SOP:

5. Definitions: N/A

6. Precautions:

- 6.1. Keep fingers away from rotor while pump is in operation. Stop pump before loading and unloading tubing.
- 6.2. Make sure no fluid is present in the tubing that is used for welding before performing the sterile welding.

7. Responsibilities:

- 7.1. It is the responsibility of the course instructor/ lab assistant to ensure that this SOP is performed as described and to update the procedures when necessary.
- 7.2. It is the responsibility of the students/ technicians to follow the SOP as described and inform the instructor about any deviations or problems that may occur while performing the procedure.

8. Equipment and Materials:

- 8.1. 70% Isopropanol (IPA) in spray bottle
- 8.2. Sterile Media and Bottle with Transfer Cap Assembly (0.2 µm filter with 3 inches of 16-inch tubing and sterile 36 inched length AdvantaFlex size 16 tubing (CR-TUBAD-0016))
- 8.3. PPE
- 8.4. TerumoBCT SCD 11B sterile tube welder; Catalog #: 1B03903

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- 8.5. SCD Wafers; Lot #: NC220713
- 8.6. MasterFlex L/S; Catalog #: C21004629 (pump drive) with MasterFlex easy-load 3; Model 77800-60, Cat number: B21004435 (pump head).
- 8.7. Sterile 2 L Cell Culture bag; Lot #: 005384-001
- 8.8. ChemCell rocking platform; Catalog #: 13243



9. Procedure:

- 9.1. Swab the table, bench surface, pump, and welder with 70% isopropyl alcohol.
- 9.2. Refer to Figure 1 for placement of pump head, tube welder, and rocker.
- 9.3. Mounting of cell culture bag on the Chemcell rocker.
 - 9.3.1. Flip the clamps from the inside position by lifting, not turning them to face the outside of the rocker. The clamps should be loose at this point.
 - 9.3.2. Slide the cell culture bag into the gap between the clamp and the rocker. Make sure the end rods of the bag are under the clamp holder.
 - 9.3.3. Return the clamps to the inside position. The clamps should be tight and secure at this point.
- 9.4. Mounting of feeding tube on the pump head.
 - 9.4.1. Verify the pump head is attached to the drive.
 - 9.4.2. Verify that the pump power cord is plugged in, and the pump is switched off.
 - 9.4.3. Place the media bottle with the transfer assembly adjacent to the left side of the pump drive.
 - 9.4.4. Open the pump head by holding the pump head with one hand. Then pull the black bottom right lever from the bottom to the top of the pump head with the other hand. The lever should line with the end of the track.
 - 9.4.5. Feed the 36-inch-long tubing through the pump head. Start with the unattached end of the tubing. Lead the end through the top black clamps, then down and around the metal rollers on the pump head. Pull the tubing out through the bottom black clamp on the pump head. The tubing should be in a backwards “C” shape.
 - 9.4.6. Verify that the tubing is secure in the top and bottom black clamps.
 - 9.4.7. While holding the pump head with one hand, return the black lever to the original position. There should be resistance as the lever moves across the track.
- 9.5. Setting up the welder for sterile welding.
 - 9.5.1. Turn on the welder by flipping the power switch to the “ON” position. The power switch is located on the right rear of the welder, towards the bottom of the welder.
 - 9.5.2. Verify that the wafer cartridge is in place and contains wafers.
 - 9.5.3. Open the right side of the tube holder cover then open the left side of the tube holder cover.
 - 9.5.4. Press the [✓] Button to align the tube holder. (Note: if the tube holder is aligned, no action occurs.)



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- 9.5.5. Verify the clamps are aligned, contact instructor or lab technician if the tube holders are not aligned.
- 9.5.6. Insert the tubing from the pump head on the top groove. Ensuring the tubing extends at least 1 inch beyond the edge of the tube holder.
- 9.5.7. Insert the 39-inch tubing from the cell culture bag on the bottom groove. Ensure the tubing extends at least 1 inch beyond the edge of the tube holder.
- 9.5.8. Close the tube holder covers; close the left holder first by pressing the cover down until the latch clicks into place, then repeat for the right cover. Ensure the holder covers are latched and secure.
- 9.5.9. Press the [1] button.
- 9.5.10. Move the white knob on the top of the machine all the way forward and then move back to its original position in one smooth motion. This will replace the old wafer with a new one.
- 9.5.11. Remove the old wafer from the front of the tube holder. Discard old wafer in container labeled "Old Wafer Blades".
- 9.5.12. While the red light is lit on the [1] button. Press the [2] button to begin the welding cycle. The [2] button light will come on and remain on until the welding cycle is complete.
DO NOT open the tube holder cover or advance another wafer until the [1] and [2] button lights are off.
- 9.5.13. When the [1] and [2] button lights are off, open the right tube holder cover first then the left tube holder cover.
- 9.5.14. Remove the welded tubing. Inspect the welded tube for completeness by rotating the tube. Remove excess tubing if necessary. If welding is not completed, contact instructor or lab technician.
- 9.5.15. Remove the pieces of stub ends tubing from the left rear and right front tube grooves and discard.
- 9.5.16. While the tube holder cover is open, Press the [✓] Button to realign the tube holder.
- 9.5.17. Hold the welded tubing in hand with the flatten side facing up. Open the seal by pinching the tubing on both sides of the weld until the seal opens.
- 9.5.18. Check the integrity of the weld by gently pulling the welded tubing.
- 9.5.19. Turn off the welder by flipping the power switch located on the right rear side.
- 9.6. Media transfer
 - 9.6.1. Ensure all clamps have been open on tubing.
 - 9.6.2. Turn on the pump by flipping the switch located on the rear of the pump drive.

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- 9.6.3. Confirm the display on the screen of the pump matches as below. If not set correctly, use the arrows next to the screen to go to the speed then select the  button. When selected, change the speed with the up and down arrow till they reach 40 mL/ minute. Once changed, select the  button to confirm speed.



- 9.6.4. Press the  button on the drive console to start the media transfer.
- 9.6.5. Once media is transferred and the tubing is empty, press the  button to stop the pump drive.
- 9.6.6. Turn off the pump drive by flipping the switch on the rear of the pump drive.
- 9.6.7. Close the tubing by closing the clamps.
- 9.6.8. Open the pump head by holding the pump head with one hand. Then pull the black bottom right lever from the bottom to the top of the pump head with the other hand. The lever should line with the end of the track.
- 9.6.9. Remove the tubing starting from the bottom black clamp, then removing from the top black clamp
- 9.6.10. While holding the pump head with one hand, return the black lever to the original position.
- 9.6.11. Cut the tubing to detach the media bottle from the cell culture bag.

10. Attachments

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Figure 1

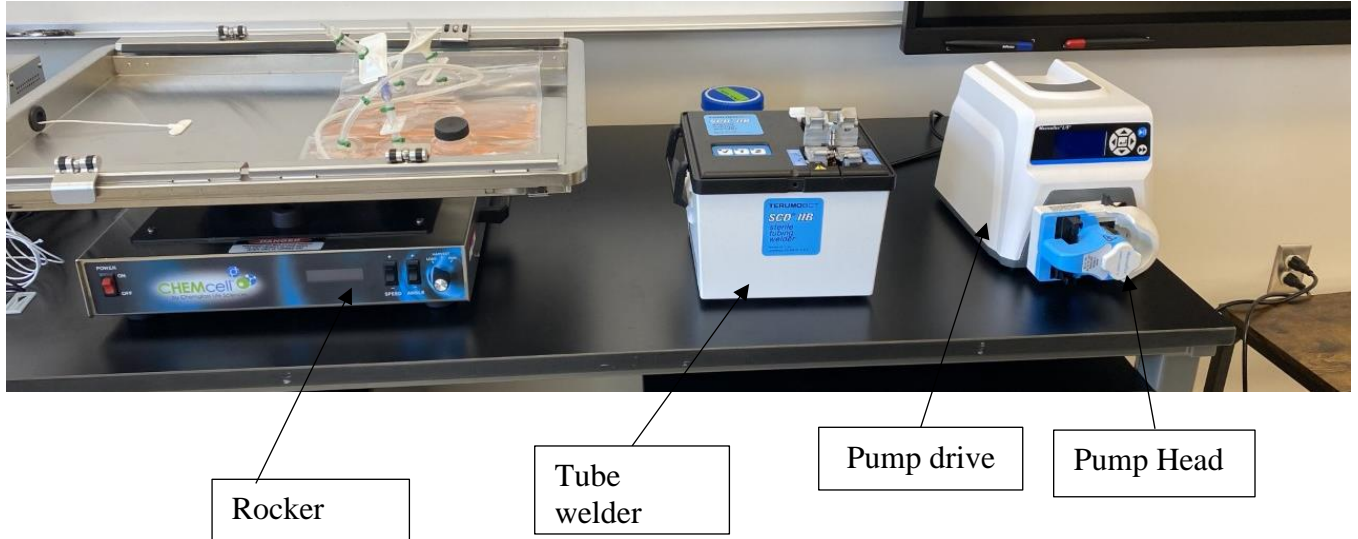


Figure 2

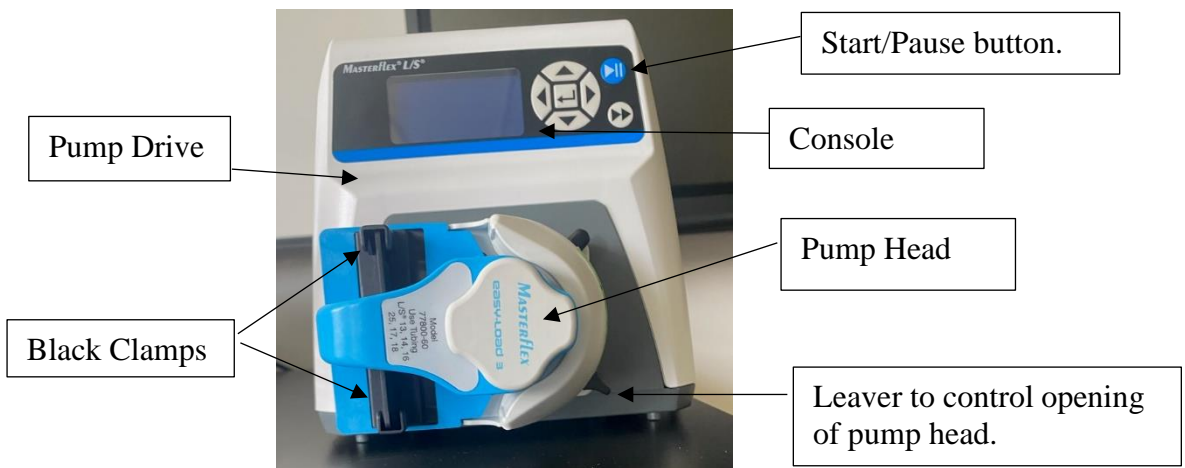


Figure 3

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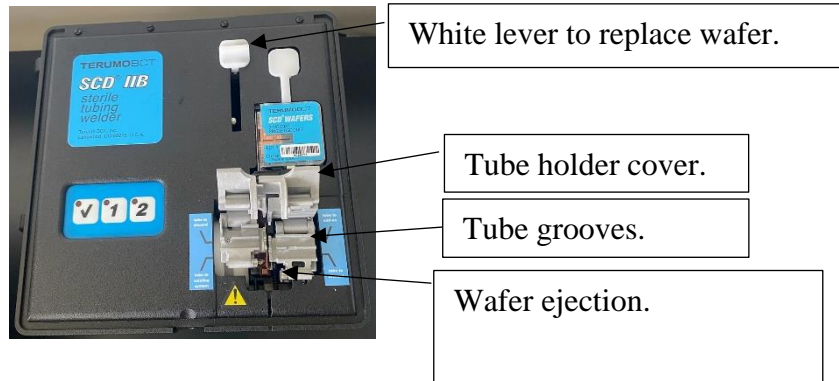
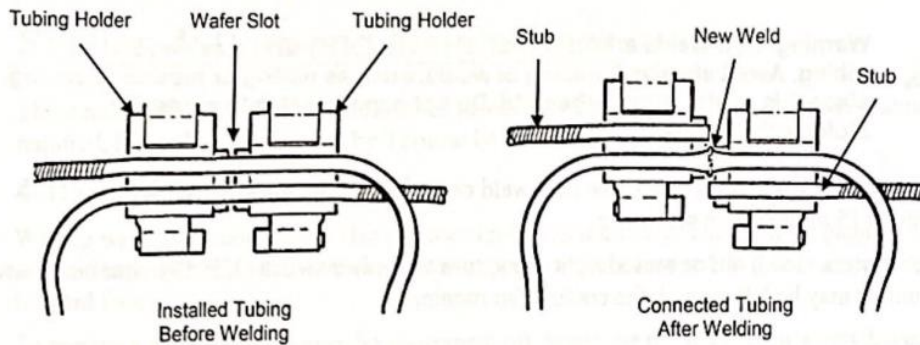


Figure 4



11. History:

| Revision Number | Effective Date | | Preparer | Description of Change |
|-----------------|----------------|--|-------------|-----------------------|
| 0 | 31JULY2023 | | Bryn Mallon | Initial Release |
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