

SOP Title: Operation, Use and Maintenance of CO₂ Incubators

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1. PURPOSE

- 1.1. The purpose of this procedure is to describe the operation, use and maintenance of a CO₂ Incubator.

2. SCOPE

- 2.1. This procedure applies to all CO₂ Incubators in the Human Papillomavirus (HPV) Serology Laboratory located at the Advanced Technology Research Facility (ATRF), room C2007.

3. REFERENCES

- 3.1. Model 3100 Series Forma Series II Water Jacket CO₂ Incubator Operating and Maintenance Manual
- 3.2. Steri-Cult Model 3307 and 3310 Series CO₂ Incubator Controlled RH with Sterilization Cycle Operating and Maintenance Manual
- 3.3. HSL_EQ_019: Use and Maintenance of the Milli-Q Integral 3 Water System
- 3.4. HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility

4. RESPONSIBILITIES

- 4.1. The Research Associate, hereafter referred as analyst, is responsible for reviewing and following this procedure.
- 4.2. The Scientific Manager or designee is responsible for training personnel in this procedure and reviewing associated documentation.
- 4.3. The Quality Assurance Specialist is responsible for quality oversight and approval of this procedure.

5. DEFINITIONS

Term	Definition
CO ₂	Carbon Dioxide
FME	Facilities, Maintenance and Engineering
HSL	HPV Serology Laboratory
REES	Rees Scientific is a provider of automated temperature monitoring systems
RH	Relative Humidity
SDS	Safety Data Sheets
Type II water	Pure / Analytical Grade, used for standard applications

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6. REAGENTS, MATERIALS AND EQUIPMENT

- 6.1. CO₂ Incubator
- 6.2. Cavicide (Warehouse, Cat # 79300360)
- 6.3. Ster-ahol (VWR, Cat # 14003-358 or equivalent)
- 6.4. Type II Water (Milli-Q, HSL_EQ_019)
- 6.5. Wypalls Paper Towel (Warehouse, Cat # 79300335 or equivalent)
- 6.6. Replacement HEPA Filter for 3100 Series (VWR, Cat # 10065-492)
- 6.7. Replacement HEPA Filter for 3307 Series (ThermoFisher, Cat # 1900160)
- 6.8. Bacharach's Fyrite® (0-20%)
- 6.9. Rubber Connector Tip / Aspirator Bulb / Saturator Tube

7. HEALTH AND SAFETY CONSIDERAIONS

- 7.1. Proper safety precautions should be taken while working in a laboratory setting. This includes, but is not limited to, proper protective equipment such as lab coats, safety glasses, closed-toe shoes, and non-latex gloves.
- 7.2. Refer to the respective SDS when working with any chemicals.
- 7.3. Refer to "HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility" regarding waste disposal processes at the ATRF.

8. OPERATION AND MAINTENANCE

- 8.1. General Use of the CO₂ Incubator
 - 8.1.1. The incubator pan is filled with sterile Type II water when in use. The incubator pan can remain dry when the incubator is not being used for active experiments.
 - 8.1.2. Make sure the humidity water bottle is filled with Type II water.
 - 8.1.2.1. Open the outer chamber door, below the control board.
 - 8.1.2.2. Remove the lid of the humidity bottle and fill with sterile Type II water.

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8.1.2.3. Install the lid and close the door, being careful not to pinch the tubing.

8.1.3. The incubators are monitored by the REES System for temperature.

8.1.4. An entry is made on "HSL_EQ_002.02: Incubator Maintenance Form" if any unknown temperature excursions occur and there is potential impact to an experiment.

8.1.5. If the incubator is not functioning within normal limits, all experiments are removed from the incubator and placed in a backup location. Record a comment on HSL_EQ_002.02. Contact FME for assistance if needed.

8.1.6. See Attachment 1 for recommended incubator settings.

8.2. Weekly Maintenance

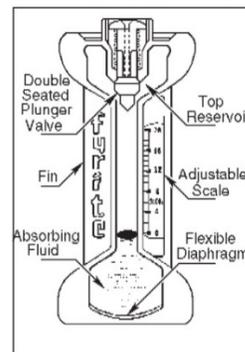
8.2.1. Once a week, measure the CO₂ level of the incubator and record the results to one decimal point on "HSL_EQ_002.01: Incubator Weekly Maintenance Form."

Note: Do not open the incubator for 2 or more hours prior to taking the measurements.

8.2.2. Preparing the Fyrite®

8.2.2.1. Refer to Figure 1 for a diagram of the Fyrite®.

Figure 1: Diagram of the Fyrite®



8.2.2.2. Handle the Fyrite® by holding it by the fin to prevent warming of fluid during analysis.

8.2.2.3. Take out the Fyrite® from the blue hard-case it came with. Fyrite® / case should be stored at room temperature when not in use.

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8.2.2.4. Check to see that a piece of wool in the Saturator Tube is wet by taking it out from the tube. If it is dried, wet with tap water.

8.2.2.4.1. Squeeze off excess water from the wool by squeezing it with your hand and padding it with a piece of paper towel.

8.2.2.4.2. Put the wetted wool back into the Saturator Tube.

8.2.3. Zeroing the Fyrite®

8.2.3.1. Hold the Fyrite® in the up-right position (away from the face; the fluid is in the bottom reservoir at this point) and press down on the Plunger Valve for a few seconds (approximately 3-5 seconds) to vent air into the device.

Note: Never vent Fyrite® in the inverted position, as this will cause the leakage of fluid from inside the device, see image below:



8.2.3.2. Release the Plunger Valve so that it closes, then invert the Fyrite® such that it is slightly at an angle to drain the fluid into the top reservoir.

8.2.3.3. Invert the Fyrite® again and hold it at ~45° angle to allow the fluid to go back into the bottom reservoir.

8.2.3.4. Hold the Fyrite® at an up-right and straight position, and allow the fluid to settle, and press down on the Plunger Valve again for a few seconds (approximately 3-5 seconds).

8.2.3.5. The level of the fluid should match to where the "0" position on the Adjustable Scale is. The scale is attached to one of four fins.

8.2.3.6. If necessary, loosen the lock that is holding the Scale to bring the "0" to match where the top of the fluid level is.

8.2.3.7. Make sure when reading and adjusting the Fyrite®, it is on a solid leveled surface.

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8.2.4. Sampling CO₂ from Incubator

8.2.4.1. Once the Fyrite® is zeroed, connect the end of the sampling tube into the "Sample Port" of an incubator.

8.2.4.1.1. The "Sample Port" on the Model 3100 Series Forma Series II Water Jacket CO₂ Incubator is located behind the outside door of the incubator (same surface level as the glass door).

8.2.4.1.2. The "Sample Port" on the Steri-Cult Model 3307 Series CO₂ Incubator is found on the upper left corner and indicated as "Sample Port."

8.2.4.2. Put the Rubber Connector Tip on top of the Plunger Valve, and press down on the valve as before, and hold. The Connector allows the air to flow into the Fyrite®; thereby allowing the sample to be collected.

8.2.4.3. While the valve is depressed, squeeze/release the aspirator bulb 18- 20 times.

8.2.4.4. After the last squeeze, while the bulb is deflated, release the Rubber Connector from the Plunger Valve.

8.2.4.5. Invert the Fyrite® to allow the fluid to drain into the top reservoir as before. Then, invert the Fyrite® again to the up-right position to let the fluid drain into the bottom reservoir.

8.2.4.6. Repeat step 8.2.4.6 one additional time.

8.2.4.7. Hold the Fyrite® at a 45° angle to allow the fluid to drain into the bottom reservoir.

8.2.4.8. Hold the Fyrite® up-right and put on a stable/leveled surface. Wait for the fluid in the column of the device to settle.

8.2.4.9. Immediately, read the percentage of CO₂ by using the numbers indicated on the Adjustable Scale. The number next to the top level of the fluid should be read.

8.2.4.10. Delay in reading by 5 -10 seconds may slightly decrease the accuracy, but longer delay could cause substantial error.

8.2.4.11. Accuracy of Fyrite® is ±0.5%.

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- 8.3.1. The CO₂ Incubators need to be cleaned every 3 months, or sooner if a spill occurs or if bacterial/fungal growth is suspected.
- 8.3.2. Remove all materials and components (shelves and water pan) from the incubator.
- 8.3.3. Spray the inside of the incubator, mounting brackets, the glass door (inside and out) as well as all removed components with cavicide and allow it to sit for 3 minutes. Wipe using a clean, low-lint wipe.
- 8.3.4. Spray the inside of the incubator, mounting brackets, the glass door (inside and out) as well as all removed components with Ster-ahol then wipe dry using a clean, low-lint wipe.
- 8.3.5. Place all components back into the incubator and fill pan (when applicable) with Type II water to approximately 1" from the top of the pan.
- 8.3.6. Document that the Quarterly Maintenance was performed on HSL_EQ_002.02.
- 8.3.7. Allow incubator temperature, CO₂, and humidity to equilibrate before use.
- 8.4. Semi-Annual Maintenance
- 8.4.1. Change incubator filters semi-annually (approximately every six months) and record the filter change on HSL_EQ_002.02.
- Note:** Semi-Annual Maintenance can be performed at the same time as the Quarterly Maintenance but needs to be noted separately on the form.
- Note:** If performing a Sterilization Cycle (section 8.5), do not replace HEPA filters until AFTER cycle has completed.
- 8.4.2. The incubators have an alarm system to indicate when filters need to be changed.
- 8.4.3. To clear the display and reset the timer after replacing the HEPA filter with a new one, perform the following steps.
- 8.4.3.1. Press the Mode Key until the Config. indicator lights up.
- 8.4.3.2. Press the right arrow until NEW HEPA is displayed in message center.
- 8.4.3.3. Press Enter to restart the timer and clear the REPLACE HEPA alarm.

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8.4.3.4. Press the Mode key to return to Run Mode.

8.5. Annual Maintenance of Steri-Cult Incubators

- 8.5.1. At least once per calendar year, or more often if needed, the Sterilization Cycle is performed on the incubator.
- 8.5.2. Perform Quarterly maintenance per section 8.3 if not already performed.
- 8.5.3. Remove HEPA filters prior to starting Sterilization Cycle.
- 8.5.4. Remove REES probe from inside the incubator.
- 8.5.5. Remove RH, CO₂, and REES sensors by pulling the sensors outward carefully. Allow them to hang on the outside of the incubator (See manual for further instructions if needed).
- 8.5.6. Install plug into sensor hole and close the access door. Press "Enter" when complete.
- 8.5.7. Press and hold the Sterilization Cycle initiation button for three seconds. The button is located on the control panel.
- 8.5.8. Confirm the HEPA filter was removed then press "Enter" on the display.
- 8.5.9. Confirm the Sensors were removed then press "Enter" on the display.
- 8.5.10. Once cycle is complete, disinfect sensors with Ster-ahol and wipe gently. Reinstall sensors.
- 8.5.11. Replace HEPA filters then hit "Enter" to return the system to "System OK" status.
- 8.5.12. Allow incubator to stabilize for at least 2 hours before use.
- 8.5.13. Document that the sterilization cycle was performed on HSL_EQ_002.02.

8.6. Calibration

- 8.6.1. FME or a contracted vendor calibrate the incubators every year as required, for routine use.
- 8.6.2. The incubators are re-calibrated if they are moved more than 10 feet.
- 8.6.3. The incubators are assessed for recalibration after repair, damage, or if physical, or electronic changes occur that could impact the operation, range,

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accuracy, or tolerance of the equipment. This is determined by the Scientific Manager or designee.

9. ATTACHMENTS

- 9.1. Attachment 1: Recommended Incubator Settings
- 9.2. Attachment 2: HSL_EQ_002.01: Incubator Weekly Maintenance Form
- 9.3. Attachment 3: HSL_EQ_002.02: Incubator Maintenance Form

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Attachment 1: Recommended Incubator Settings

Temperature: 37.0°C ± 2°C

CO₂: 5.0% ± 2%

RH: 85% ± 5% (if applicable to incubator)

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Attachment 2: HSL_EQ_002.01: Incubator Weekly Maintenance Form

Frederick National Laboratory for Cancer Research <i>sponsored by the National Cancer Institute</i>		HPV Serology Laboratory Standard Operating Procedure Form	
Form Title: CO ₂ Incubator Weekly Maintenance Form			
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Date	Initials	Equipment ID	% CO ₂	Comments
		HSL_023		<input type="checkbox"/> N/A
		HSL_024		
		HSL_026		
		HSL_027		
		HSL_023		<input type="checkbox"/> N/A
		HSL_024		
		HSL_026		
		HSL_027		
		HSL_023		<input type="checkbox"/> N/A
		HSL_024		
		HSL_026		
		HSL_027		
		HSL_023		<input type="checkbox"/> N/A
		HSL_024		
		HSL_026		
		HSL_027		
		HSL_023		<input type="checkbox"/> N/A
		HSL_024		
		HSL_026		
		HSL_027		
Reviewed by/date:				

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Attachment 3: HSL_EQ_002.02: Incubator Maintenance Form

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Maintenance Year:	
Equipment ID:	HSL_

Quarterly Maintenance

Quarter:	Q1	Q2	Q3	Q4
Cavicide Lot Number:				
Cavicide Expiration Date:				
Ster-ahol Lot Number:				
Ster-ahol Expiration Date:				
Performed by/date:				
Reviewed by/date:				

Semiannual Maintenance

Performed by/date:	
Reviewed by/date:	

Annual Maintenance

Performed by/date:	
Reviewed by/date:	

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Unscheduled Maintenance

Date	QE Number	Activity Performed	Recorded by/date	Reviewed by/date

QA Reviewed by/date: _____

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