SOP Title: Operation Use and Maintenance of $C0_2$ Incubators		
Document ID: 26001	Version	3.0
Page 1 of 15	Supersedes	2.0
Effective Date: 13Aug21		

Written by:		
Printed Name:	Title:	Signature/Date:

Approved by:		
Printed Name:	Title:	Signature/Date:
QA Approved by:		
Printed Name:	Title:	Signature/Date:

1. PURPOSE

1.1. The purpose of this procedure is to describe the operation, use, and maintenance of a CO_2 Incubator.

2. SCOPE

2.1. This procedure applies to all CO_2 Incubators.

3. REFERENCES

3.1. Model 3100 Series Forma Series II Water Jacket CO_2 Incubator Operating and Maintenance User Manual

3.2. Steri-Cult Model 3307 and 3310 Series CO_2 Incubator Controlled RH with Sterilization Cycle Operating and Maintenance User Manual

3.3. 10007: Non-Routine Equipment Maintenance

3.4. 10009: General Record Review

3.5. 26016: Operation, Use and Maintenance of the Water Purification Systems

3.6. 26027: Use and Maintenance of the FYRITE® Gas Analyzer

3.7. 15000: Waste Disposal at the Advanced Technology Research Facility

4. RESPONSIBILITIES

4.1. The Research Associate, hereafter referred to as Analyst, is responsible for reviewing and following this procedure, and documenting performance of equipment maintenance.

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.

4.4. Trained personnel perform equipment maintenance record review per “10009: General Record Review.”

5. DEFINTIONS

Term	Definition
As Needed Maintenance	Maintenance that is performed outside of routine maintenance but is not performed in response to equipment malfunction
CO_2	Carbon Dioxide
Non-Routine Maintenance	Maintenance that is performed in response to equipment malfunction or failure
REES	Rees Scientific is a provider of automated temperature monitoring systems
RH	Relative Humidity
Routine Maintenance	Maintenance that is performed at planned intervals to identify and prevent problems before they result in equipment failure
Type II Water	Pure/Analytical Grade, used for standard applications (Resistivity >1 MΩ-cm and TOC ≤ 50 ppb)

6. REAGENTS, MATERIALS, AND EQUIPMENT

6.1. Benchtop Paper (FNLCR Warehouse, Cat # 66401352 or equivalent)

6.2. Primary Disinfectant (Cavicide, FNLCR Warehouse, Cat # 79300360 or equivalent)

6.3. CO_2 Incubator

6.4. Forceps (VWR, Cat # 82027-434 or equivalent)

6.5. Gas Analyzer, FYRITE® (CO_2: 0-20%) (26027: Use and Maintenance of the FYRITE® Gas Analyzer)

6.6. HEPA Filter for 3100 Series (VWR, Cat # 10065-492)

6.7. HEPA Filter for 3307 Series (ThermoFisher, Cat # 1900160)

6.8. Preventive maintenance kit for 3307 Series (door gasket, HEPA filter, CO2 air filter, RH bacterial filter, access port filter, water bottle air filter Cat # 2270102)

6.9. Filter Replacement Kit for 3100 Series (Cat # 1900067)

6.10. Air Sample Filter for 3100 Series (Cat # 770001)

6.11. Secondary Disinfectant (Ster-ahol, VWR, Cat # 14003-358 or equivalent)

6.12. Water, Type II (Serology Laboratory Water Systems)

6.13. Wipe, Low-Lint, Wypalls (FNLCR Warehouse, Cat # 79300335 or equivalent)

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 Safety Data Sheet (SDS) when working with any chemicals.

7.3. Refer to “15000: Waste Disposal at the Advanced Technology Research Facility,” “EHS-WM-1: Disposal and Minimization of Chemical Waste,” and “EHS-WM-2: Biological Waste Handling and Disposal” for waste disposal processes.

7.4. The CO_2 Incubator has an internal environment that is sealed off from the external laboratory environment. When opening the outer Incubator door there is a glass door within to allow for viewing samples inside while minimizing contamination and environmental impact.

7.5. Incubators are not used with flammable or toxic materials.

7.6. Sterilization Cycle, if applicable, will heat Incubator’s internal surfaces, inside outer door, to 140°C. Ensure proper signage is placed on the outer door stating that the sterilization process is occurring and not to come into contact with any of the Incubator’s interior surfaces during this process.

7.7. Cavicide is typically used as the primary disinfectant cleaner. When cleaning with Cavicide, allow a minimum 3-minute contact time before wiping with a low-lint wipe. If Cavicide is not available for use, the Scientific Manager provides substitute and guidance for use.

7.8. Ster-ahol is never used as a primary disinfectant cleaner. Ster-ahol is used as a secondary cleaning agent to remove precipitate or residue left behind from Cavicide or other primary disinfectant cleaner. Ster-ahol must be sprayed on low-lint wipe then use wipe to clean surfaces. Never spray Ster-ahol directly on internal components.

8. OPERATION AND MAINTENANCE

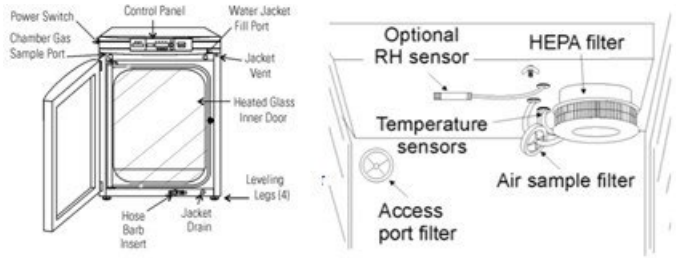
8.1. General Use of the CO_2 Incubator

8.1.1. The CO_2 levels within Incubator chamber are affected by humidity level. Ensure Incubators have access to Type II Water to allow for humidity regulation.

8.1.1.1. 3100 Series Incubator: fill Incubator Pan with sterile Type II Water to approximately 1” from top, and place Pan inside chamber under bottom shelf.

Note: Incubator Pan can remain dry when Incubator is not in use, and can be autoclaved.

Image 1: Incubator Components

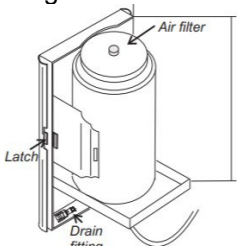


8.1.1.2. 3307 Series Incubator: fill humidity Water Bottle with Type II Water.

8.1.1.2.1 Open external door.

8.1.1.2.2 Open latch of outer chamber door, below control board. See Image 2.

Image 2: 3307 Series Incubator Water Bottle



8.1.1.2.3 Remove lid of the humidity bottle and fill with sterile Type II Water.

8.1.1.2.4 Replace lid and close both doors, being careful not to pinch tubing when closing outer chamber door.

8.1.2. The Incubators are monitored by REES System for temperature. See Settings (Section 9.)

8.1.2.1. See Attachment 1: “26001-01: Incubator Weekly Maintenance Form” for typical Incubator settings.

8.1.3. If Incubator is not functioning within normal limits, all experiments are removed from the Incubator and placed in a backup location. See Non-Routine Maintenance (Section 8.7) Contact FME for assistance if needed.

8.1.3.1. An entry is made in the appropriate laboratory assay logbook/notebook to describe the potential impact to an experiment.

8.2. As Needed Maintenance

8.2.1. If a spill occurs or if bacterial/fungal growth is suspected, clean Incubator by performing Quarterly Maintenance, see Section 8.4.

8.2.2. Document As Needed Maintenance in its respective section on form “26001-02: Incubator Maintenance Form.”

8.3. Weekly Maintenance

8.3.1. Once a week, measure CO_2 level using FYRITE® Gas Analyzer. Refer to “26027: Use and Maintenance of the FYRITE® Gas Analyzer” for use of Gas Analyzer and CO_2 sampling.

8.3.1.1. Record CO_2 level results to one decimal point on 26001-01.

8.3.2. Refill Type II Water in Incubator per step 8.1.1. Record maintenance on 26001-01.

8.4. Quarterly Maintenance

8.4.1. Clean CO_2 Incubators at least every 3 months, or more often as needed.

8.4.2. Remove all materials and components (Shelves, Water Pan, and Shields held by Wingnuts) from Incubator. See Incubator User Manual for further instructions if needed.

8.4.3. Spray primary disinfectant on low-lint wipe and wipe down insides of the Incubator, Mounting Brackets, the Glass Door (inside and out), as well as all removed components (but not Sensors).

8.4.4. Allow to sit for recommended time as per primary disinfectant guidance. Wipe clean using low-lint wipe.

8.4.5. Spray secondary disinfectant on low-lint wipe and wipe down insides of Incubator, Mounting Brackets, the Glass Door (inside and out) as well as all removed components (including Sensors).

8.4.6. Allow to sit until dry.

8.4.7. Place all components back into Incubator and refill Water Pan with Type II Water per step 8.1.1.

8.4.8. Document Quarterly Maintenance 26001-02.

8.4.9. Allow Incubator temperature, CO_2, and humidity to equilibrate before use.

8.5. Semi-Annual Maintenance

Note: Semi-Annual Maintenance can be performed at the same time as Quarterly Maintenance and Annual Maintenance but needs to be noted separately on 26001-02.

Note: If performing a Sterilization Cycle of the Steri-Cult Incubators (section 8.5, Annual Maintenance), remove old HEPA Filters and do not replace until AFTER Sterilization Cycle has completed. The filtering material cannot withstand the 140°C heat and will melt onto shelves.

8.5.1. Change Incubator Filters semi-annually (approximately every six months).

8.5.1.1. The Incubator alarms to indicate filters need to be changed.

8.5.1.2. Refer to Incubator User Manual for instructions to remove HEPA filter as needed.

Note: The 3100 Series Incubator requires the Air Sample filter replacement at the same time as the HEPA filter.

8.5.2. Clear display and reset timer after replacing HEPA Filter.

8.5.2.1. Press Mode Key until Config. indicator lights up.

8.5.2.2. Press right arrow until NEW HEPA is displayed in message center.

8.5.2.3. Press Enter.

8.5.2.4. Press Mode key to return to Run Mode.

8.5.3. Discard HEPA Filter into red-bagged biohazard box.

8.5.4. Record maintenance on 26001-02.

8.6. Annual Maintenance of Steri-Cult 3307 Incubator

8.6.1. The Sterilization Cycle is performed once per calendar year, or more often if contamination is found or when instructed by Scientific Manager.

8.6.1.1. The Sterilization Cycle requires approximately 16 total hours to complete once initiated.

8.6.1.2. Sterilization Cycle affects temperature of Incubators that are stacked, but not those adjacent.

8.6.1.2.1 Do not use secondary Incubator for an experiment if stacked with a unit undergoing Sterilization Cycle.

8.6.1.2.2 Stacked Incubators can undergo Sterilization Cycles simultaneously.

8.6.2. Verify a HEPA Filter Replacement Kit is available for use prior to performing annual maintenance.

8.6.3. Perform Quarterly maintenance per section 8.4 if not already performed.

8.6.4. Remove HEPA Filters prior to starting Sterilization Cycle.

8.6.5. Log into REES system and inhibit Incubator for 48 hours. Add ‘Annual Maintenance’ comment in system.

8.6.6. Remove REES probe from inside Incubator.

8.6.7. Remove RH, CO_2, and REES sensors by pulling the Sensors outward carefully. Allow them to hang on the outside of Incubator (See User Manual for further instructions if needed).

Note: Do not saturate Sensors or immerse Sensors in a cleaner.

8.6.8. Install plug into sensor hole and close the access door. Press “Enter” when complete.

8.6.9. Press and hold Sterilization Cycle initiation button on control panel for three seconds until initiation button illuminates.

8.6.10. Confirm HEPA Filter was removed, then press “Enter” on the display.

8.6.11. Confirm Sensors were removed, close Incubator glass door and external door, then press “Enter” on the display.

8.6.12. Place “HOT” warning sign on incubator indicating the sterilization cycle is in progress.

8.6.13. Once cycle is complete, disinfect Sensors with Ster-ahol and wipe gently. Reinstall Sensors.

8.6.14. Install new HEPA Filters then hit “Enter” to return system to “System OK” status.

8.6.15. Allow Incubator conditions to stabilize for at least 2 hours before use.

8.6.16. If Incubator is put back into service prior to the REES 48-hour inhibition is over, reactivate the Incubator by logging into REES system.

8.6.17. Document Sterilization Cycle and Semi-Annual Maintenance (replacement of HEPA filter) was performed on 26001-02.

8.7. Non-Routine Maintenance

8.7.1. In the case that the Incubator is not operating correctly, transition processes being performed to another unit (when applicable), post a sign stating the equipment is out of service and initiate non-routine maintenance documentation per “10007: Non-Routine Equipment Maintenance.”

8.7.2. Document the nature of any failures or malfunctions, how and when it was discovered, and the personnel involved on “10007-01: Non-Routine Equipment Maintenance Form.”

8.7.3. Initiate a service request and complete the non-routine maintenance process following 10007.

8.8. Annual Calibration

8.8.1. Facilities, Maintenance, and Engineering (FME) or a contracted vendor calibrate Incubators every year as required, for routine use.

8.8.2. Incubators are re-calibrated if moved more than 10 feet.

8.8.3. Incubators are assessed for recalibration after repair, damage, or if physical, or electronic changes occur that could impact the operation, range, accuracy, or tolerance of the equipment. This is determined by the Scientific Manager or designee.

9. SETTINGS

9.1. Temperature: 37.0°C ± 2°C

9.2. CO2: 5.0% ± 2%

9.3. RH: 85% ± 5% (if applicable to Incubator)

9.4. Out of Range Events

9.4.1. If REES system or Incubator goes into alarm, acknowledge alarm by emailing the laboratory personnel and log-in to the REES system to inhibit for instrument for no more than 2 hours. If the instrument maintains a temperature, CO_2, or RH out of range for more than 2 hours, then transfer biological contents to another unit. Initiate non-routine maintenance per section 8.7.

10. ATTACHMENTS

10.1. Attachment 1: 26001-01: Incubator Weekly Maintenance Form

10.2. Attachment 2: 26001-02: Incubator Maintenance Form

11. REVISION HISTORY

Version	Change	Reason
1.0	Create New SOP for the operation, use and maintenance of the CO2 Incubators.	Currently no SOP.
2.0	Updated to new template. Forms now separate. Grammar revisions throughout document. Deleted HSL_GL_002, HSL_GL_003, HSL_GL_006, HSL_GL_007, HSL_GL_008, HSL_GL_009, HSL_GL_010 and added HSL_EQ_019 to References section. Added Type II Water to Reagents section. Added REES, RH and Type II Water and removed ATRF, HSL, HPV and SOP from Definitions section. Added Note and additional instruction for finding sample port to Weekly Maintenance section. New form HSL_EQ_002.02.	Consistency between procedures; ease of use. Clarification. Deleted references not in body of procedure; added reference in body of procedure. Consistency between procedures. Deleted definitions not in body of procedure; added definitions in body of procedure. Clarification. Ease of use; maintenance easier to track and document.
3.0	3.10 updated 15000 SOP number 3.3 Added new SOP reference 3.4 Added new SOP reference Section 6 added air sample filter 8.5.1.2 Added note for air sample filter replacement 8.6.12 added section to notify sterilization cycle is in progress 8.7 Non Routine Maintenance added	Reflect current practices Reflect current practices Reflect current practices Manual reference of material Manual instructions Safety measure added Reflect current practices

Attachment 2: HSL_EQ_002.01: Incubator Weekly Maintenance Form

Frederick National Laboratory for Cancer Research — sponsored by the National Cancer Institute		Vaccine, Immunity and Cancer Directorate Standard Operating Procedure Form	
Form Title: CO_2 Incubator Weekly Maintenance Form			
Document ID: 26001-01		Version:	3.0
Associated SOP: 26001		Effective Date:	13Aug21
Supersedes:	2.0	**Page 1 of 1**	

Month / Year (MMM YYYY)				
Incubator 1 ID: (Top)			Incubator 2 ID: (Bottom)	
Date	**Initials**	**Incubator Number**	**% CO_2 (5.0 ± 2%)**	**Comments**
		1	☐ N/A ☐ Not In Use	☐ Type II Water added ☐ N/A
		2	☐ N/A ☐ Not In Use	
☐ N/A		1	☐ N/A ☐ Not In Use	☐ Type II Water added ☐ N/A
		2	☐ N/A ☐ Not In Use	
☐ N/A		1	☐ N/A ☐ Not In Use	☐ Type II Water added ☐ N/A
		2	☐ N/A ☐ Not In Use	
☐ N/A		1	☐ N/A ☐ Not In Use	☐ Type II Water added ☐ N/A
		2	☐ N/A ☐ Not In Use	
☐ N/A		1	☐ N/A ☐ Not In Use	☐ Type II Water added ☐ N/A
		2	☐ N/A ☐ Not In Use	
Reviewed by/date:				

Verify current version prior to use. Use of a superseded or obsolete document is prohibited.

This document contains confidential and proprietary information. Do not copy or distribute without prior, written permission.

Attachment 3: HSL_EQ_002.02: Incubator Maintenance Form

Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute		Vaccine, Immunity and Cancer Directorate Standard Operating Procedure Form	
Form Title: CO2 Incubator Maintenance Form			
Document ID: 26001-02		Version:	3.0
Associated SOP: 26001		Effective Date:	13Aug21
Supersedes:	2.0	**Page 1 of 2**	

Maintenance Year: (YYYY)	
Equipment ID:	

Quarterly Maintenance

Quarter:	Q1	Q2	Q3	Q4
Primary Disinfectant Lot Number:				
Primary Disinfectant Expiration Date:				
Secondary Disinfectant Lot Number:				
Secondary Disinfectant Expiration Date:				
Type II Water Refilled: (√)				
Performed By/Date:				
Reviewed By/Date:				

Semiannual Maintenance

Performed By/Date:		
Reviewed By/Date:		

Annual Maintenance ☐ N/A Section, 3100 Series; maintenance not performed

Sterilization Cycle Completed:	☐ N/A ☐ Yes
Performed By/Date:	
Reviewed By/Date:	

Verify current version prior to use. Use of a superseded or obsolete document is prohibited.

This document contains confidential and proprietary information. Do not copy or distribute without prior, written permission.

Frederick National Laboratory for Cancer Research
sponsored by the National Cancer Institute

Vaccine, Immunity and Cancer Directorate
Standard Operating Procedure Form

Form Title: CO2 Incubator Maintenance Form

Document ID: 26001-02		Version:	3.0
Associated SOP: 26001		Effective Date:	13Aug21
Supersedes:	2.0	**Page 2 of 2**	

As Needed Maintenance: ☐ N/A

Date	Activity Performed	Recorded By/Date	Reviewed By/Date
☐ N/A			
☐ N/A			
☐ N/A			
☐ N/A			

Verify current version prior to use. Use of a superseded or obsolete document is prohibited.

This document contains confidential and proprietary information. Do not copy or distribute without prior, written permission.