Maintenance in the Microbiology Lab

Purpose

This procedure provides instruction for monthly, bi-monthly, quarterly, semi-annual and annual maintenance assigned to Desk 3 in the Microbiology Lab. The procedures listed may be completed at any time provided they are completed before the end of each month, bi-monthly or quarterly period.

Principal and Clinical Significance

Clean equipment helps ensure a safe environment and minimized biohazard exposure to laboratory staff.

Policy Statements

This procedure applies to Microbiologists and technicians that work in the Microbiology lab.

Materials

Reagents	Supplies	Equipment
• DI H2O • 70% alcohol solution • Liquinox - located under the rapid molecular sink • Biocidal ZF spray -located under the rapid molecular sink • Pluronic water tube • Petroleum Jelly	• PPE: lab coat, gloves, safety glasses • Linen towel(s) - located above printer by Processing • Oxivir wipes • Forceps • Absorbent-wipes or other lint free cloth • MicroScan Seed trough • MicroScan Cover tray	•Drill Bits numbered 53 and 54 •Teflon lubricant spray – located above the Anoxomat instrument

Special Safety Precautions

Microbiologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology Procedure Manual*.

- *Biohazard Containment*
- *Biohazardous Spills*
- *Safety in the Microbiology Laboratory*

Monthly Maintenance Procedure

Urine Loop Calibration

1. At the culture desk hood, obtain the #53 and #54 drill bits stored in the plastic case in the cart adjacent to the BSC. They are labeled with small pieces of tape: “GO” or “NO GO.”
2. Visually examine each urine loop for damage. If the loop is no longer round, it may not measure the correct volume and should be discarded.
3. Working with a single 0.001mL quantitative loop, pass the smooth end of the drill bit #54 through the loop. It should slide easily through the loop with no resistance.
4. If you are unable to slide the bit through the loop, discard the current loop and repeat the test with a new loop.
5. Repeat step 3 using drill bit #53. It should not be possible to pass the smooth end of the bit through the loop.
6. If the #53 drill bit passes through the loop at all discard the current urine loop and repeat steps 3 and 4 with a new loop.
7. Record the results in the Weekly KB, 30 day, Day of Use QC Spreadsheet in SharePoint.

8. Record completion on the Desk 3 maintenance checklist.
9. Refer to MC 8.4 Loop Calibration for further instructions.

Check Fyrite Strength

1. Before taking a CO_2 reading, vent the Fyrite to release any residual gas and allow fluid level to normalize.
2. Check that the fluid meniscus measures at zero. If the fluid is above or below the zero marking, adjust the ruler accordingly by loosening the silver screw holding the ruler in place and slide up or down as needed. Tighten the screw.
3. Invert the Fyrite for one cycle, allowing the fluid to drain first into the top reservoir, and then holding it upright, to drain into the bottom reservoir. Now the Fyrite is ready to take a CO_2 reading.
4. Attach the open rubber tubing to the gas porthole of a CO_2 incubator and squeeze the bulb three times to prime.
5. Place the sampling assembly rubber connector tip over the plunger valve and depress firmly. Keeping the plunger depressed, pump the blue bulb 18 times to sample CO_2 concentration.
6. Remove sampling assembly ensuring that the plunger is no longer depressed. Invert Fyrite allowing all fluid to drain into the top reservoir.
7. Hold Fyrite upright and allow all fluid to drain into the bottom reservoir. Momentarily tipping Fyrite to a 45° angle will allow any remaining droplets to fall to the lower reservoir. Read the gauge and record the value.
8. Do not vent the reservoir. Instead complete an additional inversion cycle by inverting to allow fluid to drain into the top reservoir and then turn upright, allowing fluid to drain to the bottom reservoir.
9. Read the gauge.
10. If the second reading increases by over half a percent, then the Fyrite fluid must be changed. See example given below.
    a) First reading: 5.0% CO_2. Second reading: 6.5% CO_2—CHANGE FLUID
    b) First reading: 5.0% CO_2. Second reading 5.5% CO_2-- FLUID OK
11. Vent the Fyrite before storing.
12. Record on the Desk 3 maintenance checklist.
13. Refer to MCVI 6.7 Fyrite CO_2 Measurement for further instructions.

Clean Cytocentrifuge

1. Clean outside surfaces and switch overlay panel with a water-dampened cloth and mild detergent.
2. Clean the inner surface or bowl, a powder-coated steel surface, with a mild detergent and disinfect if necessary, by wiping with a cloth dampened with 70% alcohol or 10% bleach.
3. IMPORTANT: DO NOT SPRAY the bowl or outer surfaces with detergent or bleach.
4. If there is an instrument malfunction, document failure. Contact Biomed by filing an online Soleran report. Notify Microbiology Technical Specialist.
5. Refer to MCVI 6.1 Cytocentrifuge.
6. Record on the Desk 3 Maintenance Checklist.

Clean Centrifuges

To clean the Heraeus Megafuge (used for OAPs)

1. Follow steps 1-4 above under Cytocentrifuge.
2. Remove all buckets and bucket inserts.

3. Disassemble the inserts and wipe down each component with Oxivir wipe(s).
4. Once dry, return inserts and buckets to the centrifuge.
5. Record on the Desk 3 Maintenance Checklist.

To clean the Heraeus Virology Centrifuge:

1. Wipe down the exterior.
2. Because this centrifuge is rarely used, there is a piece of tape marking the lid. If the tape has not been removed or otherwise altered, the centrifuge does not require cleaning. If it has been removed, wipe out the bowl, buckets, and inserts as described above.
3. Record on the Desk 3 Maintenance Checklist.

Clean Hoods

Note: This procedure applies to both the hood located at culture desk and the two hoods in Rapid Molecular.

1. Begin by turning off the incinerator and slide heater. Once cool enough to handle without risking injury or fire, wipe down all materials contained in the hood with an Oxivir wipe (loops, vortex, incinerator, tube rack etc.).
2. Remove all materials from the hood and set aside.
3. To gain easier access to hood surfaces, temporarily disable the window alarm to lift the window glass. Do NOT deactivate the blower/exhaust fan.
4. There are screws located on each side of the hood near the grates. They are hand-tightened. Remove the screws but do not lose them.
5. The metal work surface may be lifted or removed completely to clean the inner catch and underside of the hood. It is heavy—take care when lifting.
6. Because the culture desk hood frequently collects a lot of dust, it is often easier to wipe down the inner catch, (underside of main worksurface) of the BSC with a Swiffer dry sweeper cloth to remove dust before attempting to clean further.
7. Remove the grate to clean it.
8. Using either an Oxivir wipe for the culture desk hood, or a bleach wipe for the molecular hoods, wipe down all surfaces of the BSC including the underside of the main worksurface and the inner catch.
9. Repeat step eight using DI water on a low-lint absorbent cloth followed by a solution of 70% ethanol.
10. Once the hood is dry and the work surface has been properly replaced, return all materials to the hood as needed. Lower the window sash to the appropriate height if it was adjusted during cleaning and ensure the window alarm buzzer is activated.
11. Record on Desk 3 Maintenance Checklist.
12. Refer to SA 10.7.3 Cleaning Laboratory Equipment

Clean Slide Heater

1. Switch off the slide heater and allow it to cool to the touch before proceeding.
2. Using an Oxivir wipe, wipe the outer surfaces of the heater.
3. With the same wipe, clean each slot on the heater. Use forceps to help reach corners and crevices.
4. Dispose of soiled wipe(s) in a biohazard bin.
5. Upon completion, turn on the slide heater and check periodically to ensure the temperature returns to between 58°C and 62°C.
6. Record on the Desk 3 maintenance checklist.

Clean Incubator Water Trays

NOTE: This procedure occurs monthly but is readily combined with bi-monthly incubator cleaning when required.

1. Lay cloth towels on a rolling cart or on the floor at the base of each incubator stack.
2. Carefully remove the humidifying pan cover and the metal water tray from the bottom of the incubator to be cleaned.
3. Slowly pour the water out of the incubator tray and into the yellow bucket.
4. Note: Transferring water from the incubator tray into the yellow bucket is not required, but it does help prevent spills. The plastic graduated cylinder by the rapid molecular sink can also be used to remove some water from the tray before attempting to empty the tray fully.
5. Repeat with the remaining water trays until all four are empty.
6. Working with one tray at a time, use an absorbent-wipe or paper towel to dry residual water from the tray.
7. Using the biocidal spray, lightly mist each tray. Do not wipe. Allow to air-dry. Warning: Biocidal spray is an irritant—avoid contact with eyes and mucosa.
8. Once all trays are dry, refill each one with STERILE distilled water. Deionized or reverse-osmosis water leads to corrosion and should not be used. Wipe up any water spills to further prevent rusting.
9. Record on Desk 3 Maintenance checklist.

FA Scope Hours

1. Using the black dial next to scope, press down and hold until the lamp intensity percentage is replaced with a menu.
2. Using the scroll wheel, highlight “SRVC” and select it by pressing down on the dial.
3. Scroll to find “Hour” and select it. This is the FA scope hour reading.
4. Record value on Desk 3 monthly maintenance checklist. If the value is above 20,000 hours, the bulb must be replaced.
5. See MCVI 6.7 Fluorescent Microscope for further information if necessary.

Clean MicroScan Cover Trays

1. Fill the yellow specimen bucket with Liquinox at a 1:100 ratio with tap water.
2. Allow MicroScan trays to soak in solution for one minute.
3. Rinse soap from MicroScan cover trays thoroughly with deionized water and allow to air-dry completely before storing.
4. The remaining Liquinox solution may be disposed of in the sink.
5. Record on Desk 3 Maintenance Checklist.

MicroScan Dispenser Check

1. Obtain the scale located at Desk 3 in the drawer containing MicroScan supplies.
2. Obtain MicroScan seed trough, cover tray and tube of Pluronic.
3. Turn on the scale, place the cover tray on the scale, and zero the scale.
4. Remove transfer lid of seed trough. Inoculate the seed trough with the tube of Pluronic water. Rock the trough to distribute the inoculum evenly through the trough.
5. Replace transfer lid over seed trough. Gently tap the transfer lid in all four corners to ensure absence of bubbles. Allow the cover tray to equilibrate for a minimum of 20 seconds.
6. Use the RENOK to transfer inoculum as follows:
    a. To pick up the RENOK unit from its stand, depress the pick-up levers at the sides using thumb and forefinger.
    b. Place the RENOK unit on top of the transfer lid of the seed trough.
    c. Fully lift the large center lever of the RENOK unit to draw up inoculum into the transfer lid.
    d. Depress the center release button to return the inoculum to the seed trough. This will prime the unit to reduce bubble formation.
    e. Fully lift the large center lever again to draw up the inoculum.
    f. Pick up the unit with the transfer lid attached, and, while holding it level, position the transfer lid onto the MicroScan cover tray.
    g. Dispense into the cover tray by pressing the top center release button,
    h. Return the lid to the seed trough.
    i. Place cover tray on scale.
    j. Weigh the filled cover tray and record the weight.
7. Weight should measure between **10.1 and 12.0 grams.**
8. Record on the Desk 3 Maintenance Checklist.
9. If the fill volume is not within the desired range, repeat the above steps if an obvious error can be determined. If no clear source of error is found, replace the rubber seal and repeat.
10. Refer to MC 6.40 MicroScan® MIC/COMBO Procedure-- RENOK Rehydrator/Inoculator Dispenser Volume Check.

Clean Gram Stain Sink

1. The sink used for Gram stains is prone to precipitate build-up and can be cleaned regularly to preserve sink basin integrity.
2. Place a drain plug or barrier in the drainage hole at the bottom of the sink to prevent cleaning solvent from dripping into the stain waste carboy.
3. Obtain a few absorbent wipes and the Erado-sol stain remover from under the Rapid Molecular sink.
4. Lightly mist the sink basin with Erado-sol stain remover and lay the absorbent wipes on top, targeting highly stained areas. Note that Erado-sol is most effective in light coatings—do NOT oversaturate.
5. Allow the absorbent wipes and Erado-sol to soak for at least five minutes.
6. Remove the absorbent wipes. Using a fresh cloth damp with water, wipe the sink basin to remove stain residue.

7. Remove the drain plug and ensure the stain-waste carboy is properly connected.
8. Record on Desk 3 Maintenance Checklist.

Bi-Monthly Maintenance Procedures

Clean Incubators

1. Move any incubating plates to the adjacent CO_2 incubator before cleaning.
2. Remove all incubator attachments including:
    a. Shelves
    b. Shelf supports
    c. Humidifying pan cover and water tray
    d. Top duct cover of circulating fan
    e. Side supports
3. Using a mild detergent solution (Liquinox diluted 1:100 in tap water), wipe down all attachments and follow with a rinse in distilled water.
4. Using Biocidal ZF incubator cleaning spray, spray the inside walls of the incubators. Allow to air dry.
5. OR, using a cloth wet with 70% ethanol or 70% isopropyl alcohol, wipe down the inside walls of the incubator. Follow with a dry cloth.
6. Replace all attachments. Confirm that incubator is operating within specification (temperature and %CO_2) before returning any plates to incubate.
7. Repeat as needed with remaining incubators.
8. Record on Desk 3 Maintenance Checklist.
9. Refer to SA 10.7.3 Cleaning Laboratory Equipment

Quarterly Maintenance Procedures

Anoxomat Jar Maintenance

1. Anoxomat Maintenance is scheduled quarterly, in January, April, July and October.
2. Clean jars with soap and water only. Do **not** use alcohol or bleach to clean the jars as these chemicals can damage the jar over time. Take care to wipe the rubber O-ring in each lid. Dry the jars gently with a low lint cloth or towel.
3. Examine jars for cracks or other signs of overuse. Discontinue use of any cracked jar immediately.
4. Spray a very small amount of Teflon lubricant in each coupling. Use a swab or stick to press down the center of each coupling a few times to work in the lubricant. This will maintain the snap-shut couplings on each jar lid.
5. Apply acid-free petroleum jelly to the O-rings in the standard (large, round, non-ergonomic) jars.
6. Record task on the Desk 3 Maintenance checklist.
7. Refer to MCVI 6.9 Anoxomat Instrument for further instructions.

Semi-Annual Maintenance Procedures

Change Fyrite

- Refer to MCVI 6.8 Fyrite CO_2 Measurement for instructions on how to change the Fyrite fluid
- Record on Desk 3 Maintenance Checklist

Annual Maintenance

Calibrate Thermometers

- Record results in Thermometer binder
- Refer to CH 2.10 Thermometer Calibration for instructions.
- Record on Desk 3 Maintenance Checklist

Calibrate Pipettes

- Pipettes are calibrated by an outside vendor annually.
- Record on Desk 3 Maintenance Checklist

References

MC 6.40 MicroScan MIC/COMBO Procedure
MC 8.4 Loop Calibration
MCVI 6.1 Cytocentrifuge
MCVI 6.8 Fyrite CO2 Measurement
MCVI 6.9 Anoxomat
SA10.7.3 Cleaning Laboratory Equipment
Operators manual for SANYO CO_2 INCUBATOR Model MCO19AIC

Training Plan/ Competency Assessment

Training Plan	Initial Competency Assessment
1. Employee must read the procedure. 2. Employee will observe trainer performing the procedure. 3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.	1. Direct observation.

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1	Katie Bina, Vilada Louangkhot Obnamia,Sarah Wadd, Andrew Fangel, Susan DeMeyere	10/17/2024	Initial version