TIMER VERIFICATION
Culture Lab and Toxicity Incubators

1. SCOPE AND APPLICATION

1.1. These procedures are used to verify that the shaker rack cycles for AGP/algae cultures and the prescribed light/dark cycles are properly controlled in the bioassay test chambers and the bioassay organism culture areas based on requirements set forth in EPA 821-R-02-012, EPA 821-R-02-013, and EPA 821-R-02-014.

1.2. All incubator timers are set to 16 hours light and 8 hours dark (24-hour period) except those used for algal cultures, which are set for continuous lighting. Room lighting timer C is set to go off at 5:00 PM (to conserve energy).

1.3. Algae shaker rack timers are set for a duration of 1 hour twice a day (generally 8:00-9:00 AM and 3:00-4:00 PM).

2. SUMMARY OF METHOD

2.1. Current time, as indicated by timers, is compared to the correct time and adjusted if necessary. The proper functioning of the on/off switches are tested by setting the timers near on/off times and allowing them to trip the switches. The supervisor is notified of any problems.

2.2. Light timers are verified monthly, although the NELAC requirement is quarterly. Timers should also be checked/reset after power failure events. Verifications and aberrations are recorded in the *Light Timer Check* spreadsheet.

2.3. Daily observations of incubators (in use) and the culture laboratory are made and any deviations from the scheduled cycle (lights off when scheduled to be on, etc.) should be pointed out and reported as necessary, and corrective action taken immediately.

3. EQUIPMENT AND SUPPLIES

3.1. *Light Timer Check* spreadsheet (located in B246)

3.2. Dayton Model 4A214, 24-hour timer (Lab ID Timer A)

3.3. Dayton Model T1472BR, 24-hour dial timer (Lab ID Timer B)

3.4. Intermatic Model T173, 24-hour dial timer (Lab ID Timer C)

3.5. Intermatic Model ET70815CR, Programmable Timer (Lab ID AGP Timer)

3.6. Thermo-Scientific Incubator (Precision Model 818) #1, #4, #5, and #7

3.7. Thermo Scientific 120V Incubator #8

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3.8. MRC LOM-150-LIGHT Incubator #9

4. PROCEDURE

4.1. **Mechanical Timers A, B, and C**

4.1.1. Mechanical timers A and B are located on the west wall of B246; mechanical timer C is in the southeast corner of B246.

4.1.2. Ensure that the time piece is within ±1 minute of the correct time. Time can be checked with another time piece, computer, smart phone, etc. The U.S. Naval Observatory Master Clock (https://www.usno.navy.mil/USNO/time/display-clocks/simpletime) is a good source for the correct time.

4.1.2.1. It should be noted that the dial timers can only be set in increments of 15 minutes.

4.1.3. To verify that the timer turns off the controlled lights, grasp the edge of the circular dial, pull outwards (about 1/4 inch) and turn clockwise until the time on the dial is before 10 PM (for timers A and B) or 5 PM (for timer C). Leave the manual lever (below the dial) in the “ON” position. The controlled lights should turn off when the timer hits the designated time.

4.1.3.1. The current time the dial is reading is indicated by a stationary metal pointer located on the bottom inner rim of the dial. The set-time markers are located on the outer rim of the dial on the desired time marker. These markers move with the dial as time passes. Once the set-time markers reach the stationary current time marker, the mechanism is triggered and the lights go on or off as programmed.

4.1.3.2. Setting the dial too close to the shut off (on) time may cause it to bypass the switch and remain on (or off). It is best to set the time 30 minutes to an hour before the set-time to ensure proper engagement of the switch. If the switch doesn’t engage, retry the process giving more time before the set-time. If it still does not engage, notify the supervisor.

4.1.4. For timer C, the verification is complete once the lights are triggered to turn off, you can move to section 4.1.6

4.1.5. For timers A and B, verify the timer turns on the lights, set the timer to before 6 AM by manipulating the circular dial as described in 4.1.3. Keep the manual lever in the off position. The controlled lights should come on when the set-time is reached. (Section 4.1.3.2. applied here as well.)

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4.1.6. Once the verifications are complete, set the mechanical timer to the current time by turning the circular dial as described in 4.1.3 until the current time mark on the dial is aligned as near as possible with the stationary current time marker (15-minute increments).

4.1.7. If the timer needs adjusting, make sure that the "on" marker is set to 6:00 AM and the "off" marker is set to 10:00 PM on timers A and B. For timer C, the "off" marker is set to 5:00 PM.

4.2. **Incubators (#1, #4, #5, #7, #8, and #9)**

4.2.1. The timer for Incubator #7 and #8 (Marine AGP culture), and #9 (Freshwater AGP culture) are set to continuously on.

4.2.1.1. If Incubator #7 is repurposed for tests or *C. dubia* cultures, it can be tested following the method below.

4.2.1.2. Check that Incubator #9's shaker rack is working. Unfortunately, the way this incubator's timer is set up, it slowly drifts ahead in time. The shaker is set to run an hour twice a day but the time it runs will vary. The display will show how long it has left in its current cycle, which can help you determine when the shaker cycle will activate. Refer to the MRC LOM-150-LIGHT manual for shaker timer setting instructions.

4.2.2. Incubators #1, #4, and #5 are set for 16 hours light and 8 hours dark. Compare the time displayed on the display with the correct time. If the displayed time is not within ±5 minutes of the correct time, use the procedures found in the Precision Instruction Manual to set the display to the correct time. (Set the time after the on off checks have been done, as they require adjusting the time.)

4.2.3. Follow the procedures in the instruction manual to check that the on/off times are set for "on" at 6:00 AM and "off" at 10:00 PM.

4.2.4. Check the "OFF" time first (verification is normally done during the workday when the lights would be on). Follow procedures in the instruction manual to set the time to 9:59 PM. When the display shows 10:00 PM, the interior door lights should go off. Check this by opening the door (a small interior light will come on when the door opens but the large light unit will be off).

4.2.5. Check the "ON" time next. Follow the procedures in the manual to set the time to 5:59 AM. When the display shows 6:00 AM, the interior door lights should come on. Check this by opening the door.

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4.2.6. Record the date/time of verification on the Light Timer Check spreadsheet.

4.2.7. Reset the time on the incubator to reflect the correct time ±5 minutes.

4.3. **Intermatic Model ET70815CR, Programmable Timer (Lab ID AGP Timer)**

4.3.1. The Intermatic programmable timer is located on the east wall of B246 at the end of the AGP shaker platform. This timer controls the AGP shakers. It is set to operate for 1 hour twice daily during normal work hours (8:00 – 9:00 AM and 3:00 – 4:00 PM). Visually verify the operation of the shakers (should be on) during these periods. In case of power failure or if the displayed time is not within ± 5 minutes of the correct time, notify the lab supervisor. Refer to the Intermatic Timer instruction manual for procedures to set the time of day and on or off periods.

5. DATA ARCHIVAL

5.1. Record light timer checks in the printed Excel spreadsheet, Light Timer Check, once each monthly timer checks is completed.

5.2. Use a black, waterproof pen for all bench sheets and logbooks.

5.3. To correct raw data entries, place a single line through the incorrect or illegible entry, write the corrected entry near the error, date and initial the correction. Do not overwrite entries.

5.3.1. The corrected entry is written as near as possible to the error or in the comment box on the appropriate bench sheet.

5.3.2. If the correction requires an explanation, write a comment, and reference the error. Initial and date the explanation. Do not attempt to put all information (correction and comments) into a small space.

5.3.3. Use sequential alphabetical annotation to identify errors and corresponding comments (use A for first error and use A for corresponding explanatory comment; use B for second error, etc.).

6. QUALITY CONTROL

6.1. Daily observations of incubators (in use) and the culture laboratory are made.

6.2. Timers are verified monthly by comparing the presently displayed time on the timer to another time source to ensure it is within the acceptable range.

6.3. In case of power failure or if the displayed time is not within the acceptable range, the lab supervisor is notified, and corrective actions are taken.

7. SAFETY/HAZARDOUS WASTE MANAGEMENT

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7.1. Read MSDS information for reagent handling and disposal.

7.2. Use proper safety equipment including the lab hood or chemical ventilation system, a lab coat, safety glasses, a respirator, and latex gloves (or a suitable substitute if allergic to latex) when working with chemicals and unprocessed samples.

8. REFERENCES

8.1. FDEP Health and Safety Manual

8.2. FDEP Laboratory Quality Assurance Manual

8.3. Operating Instructions for Intermatic Model T173, 24-hour dial timer

8.4. Operating Instructions for Dayton Model 4A214, 24-hour dial timer

8.5. Operating Instructions for Dayton Model T1472BR, 24-hour dial timer

8.6. Instruction Manual for Thermo-Precision Scientific Incubator Model 818

8.7. Instruction Manual for Thermo Scientific 120V Incubator

8.8. Instruction Manual for MRC LOM-150-LIGHT Incubator

8.9. Instruction Manual for Intermatic Model ET70815CR Programmable Timer

8.10. EPA 821-R-02-012, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms 5th ed.* October 2002.

8.11. EPA 821-R-02-013, *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms 4th ed.* October 2002.

8.12. EPA 821-R-02-014, *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine Organisms 3rd ed.* October 2002.

APPENDIX OF SIGNIFICANT CHANGES

07/25/2008 Added Scope and Application, Summary of the Method, References and Appendix of Changes

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