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 laboratory 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 (8:00-9:00 AM and 3:00-4:00 PM).

2. SUMMARY OF THE 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 is tested by setting the timers near on/off times and allowing them to trip the switches. Supervisors are notified of any problems.

2.2. Light timers are verified monthly, although the NELAC requirement is quarterly. Timers should also be checked and 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 reported and corrective action taken immediately.

3. SUPPLIES AND EQUIPMENT

3.1. *Light Timer Check* spreadsheet (located in QC binder)

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

3.3. Dayton Model T1472BR, 24-hour dial timer (Lab 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

Note: Toxicity incubators are monitored per SOP TA-06.01.

4. PROCEDURE

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

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

4.1.3. 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 set as close to 9:45 PM (for timers A and B) or 4:45 (for timer C) as possible. Now, leave the manual lever in the on position. The controlled lights should turn off within 15 minutes.

4.1.4. For timer C, the verification is complete, and 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 approximate 5:45 AM (regardless of the current time) by manipulating the circular dial as described in 4.1.3. Keep the manual lever (below the dial) in the off position. The controlled lights should come on within 15 minutes.

4.1.6. Once the verifications are complete, set the mechanical timer to the current time by manipulating 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 timer hand (silver colored stationary pointer-resembling an hour hand - located at the center of the dial that points towards the bottom of the dial).

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

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

4.2.1. The timer for Incubator #7 (Marine AGP culture) is set for continuously on.

4.2.2. Incubators #1, #4, and #5 are set for 16 hours light, 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.

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 9:55-59 PM. When the display shows 10:00 PM, the interior door lights should go off. Check this by opening the door.

4.2.5. Check the 'on' time next. Follow procedures in the manual to set the time to 5:55-59 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.

4.3. **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 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*. When all monthly timer checks are completed and recorded.

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.).

5.4. If a problem occurs during sample processing, a nonconformance report (NCR) is submitted describing the problem and the corrective action.

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 current timer time 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

7.1. Read MSDS information for reagent handling and disposal.

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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. DEP Laboratory Health and Safety Manual

8.2. DEP Laboratory Quality 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. DEP SOP TA-06.01, Temperature Monitoring of Walk-in Refrigerator, Freezer and Toxicity Laboratory Incubators

8.8. Instruction Manual for Intermatic Model ET70815CR Programmable Timer

8.9. 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.

8.10. 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.

8.11. 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 CHANGES:

07/25/2008	Added Scope and Application, Summary of the Method, References and Appendix of Changes
03/13/2013	Deleted Incubator #5. Added instructions for Intermatic Model ET70815CR, Programmable Timer (Lab ID AGP Timer)
03/13/2013	Reformatted the SOP.
03/03/2014	Updated the format of the SOP.
02/04/2015	Added incubator # 5, changed server information, and formatted for 508 compliance.
10/15/2015	Updated links. Minor corrections.
10/10/2016	Clarified timer light/dark cycle.
10/06/2017	Removed incubator #2; added incubator #6.

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08/29/2018 Updated to include Incubator #7. Updated links and changed font to Calibri.

07/02/2019 Updated links and removed outdated instructions

08/31/2020 Updated wording in equipment section.

08/18/2021 Changes in section 5, updated Links.

08/12/2022 Updated links, updated references, checked grammar, removed incubator #6, and added information for rack timer checks.

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