

Incubator Basics

Principle of Operation

Microbiology incubators are designed to promote the growth of microorganisms by maintaining a constant temperature within a narrow range. There are many types of incubators available that offer an array of features. Advanced features may include carbon dioxide (CO_2) atmosphere, circulating fans, humidity controls, recording thermometers and alarm systems. Such features may provide an enhanced environment for growth of certain microorganisms, monitor current environmental conditions of the unit, or alert the user when tolerance limits are not met.

Many units are equipped with a power pilot light with the power switch, which provides a visible cue that the unit is on. Basic to all models is a temperature control device or thermostat. The thermostat should be adjustable to obtain the desired temperature. After the thermostat is adjusted, check the temperature every hour for several hours to ensure your incubator is properly regulated. It is imperative that the temperature of the incubator be allowed to completely stabilize after each adjustment. Incubators with low wattage heaters heat up slowly, so patience must be exercised when monitoring the temperature readings.

To avoid hot spots, items placed in the incubator (i.e., culture plates, candle jars) should be arranged in a manner that allows for maximum air circulation. Avoid covering shelves completely, allowing free space in corners.

**AFTER ADJUSTING THE THERMOSTAT,
MONITOR TEMPERATURE READINGS CLOSELY.**

CO_2 incubators are usually equipped with flow meters, which monitor the flow of CO_2 in the unit. An indication of 0.5 liters per minute on the flow meter will provide approximately 5% CO_2 tension within the chamber. A CO_2 concentration of 3-10% is acceptable for most microorganisms requiring this environment. Some units

also possess a rapid recovery CO_2 purge timer, which bypasses the CO_2 flow meter and allows for the rapid recovery of CO_2 tension after the door has been opened. The purge timer is also used for initial starts when the CO_2 tank has been changed.

Safety/Physical Concerns

Incubators should be located on level, solid work surfaces away from high traffic areas, drafts and temperature extremes. (A constant temperature is easier to maintain if the incubator is not placed in the direct airflow from air conditioners or heat sources.) You should also allow for free air space above and behind the unit. Care should be taken in placement of the unit to avoid "bumping" that may inadvertently alter the control settings. Shelves should be checked for levelness at initial installation and after replacement during cleaning. If a CO_2 tank is required, it should be securely chained to the wall in an upright, stationary position. The electrical cord should be routinely inspected for damage. Electrical tape must NOT be used for cord repair.

Operational Checks

Always refer to the manufacturer's Operator's Manual for specific instructions regarding use, maintenance or troubleshooting problems with your incubator. Lost or misplaced your manual? For a nominal fee, most companies will send you a new one. Just have your model and serial number handy when you place your order.

In general, there are some basic operational functions that should be documented each day of use.

Daily:

Check and record the internal temperature. For most microorganisms, the acceptable range from the desired setting is ± 1 degree Celsius. For CO_2 incubators, record the CO_2 flow rate and verify that the CO_2 source has not run low (as in the case with CO_2 tanks). A fyrite CO_2 indicator is suitable for verifying the CO_2 concentration.

Refer to your manufacturer's recommendations to ensure proper CO₂ concentration for your unit. Verify that pilot lights are cycling off and on intermittently. If not, the power supply and thermostat function should be checked. Also confirm that the door gasket has a tight seal. With the incubator door closed, try to insert a business card between the gasket and the door in any suspect areas. If a business card can be placed between the gasket and the door, replace the gasket or check the door alignment.

CAN'T FIND YOUR OPERATOR'S MANUAL?
REQUEST A NEW ONE FROM THE MANUFACTURER.

Humidity Requirements

Water is a major constituent of both broth and agar media. However, when media are incubated at temperatures used for bacterial cultivation, a large portion of water content can be lost through evaporation. Loss of water from media can be harmful to bacterial growth in two ways:

- ❑ Less water is available for essential bacterial metabolic pathways,
- ❑ A relative increase in the solute concentration of the media occurs which can "shock" and subsequently lyse bacterial cells.

Because increased atmospheric humidity enhances the growth of certain bacteria and decreased humidity can prove detrimental, assuring an appropriate humidified environment is recommended. This can be accomplished in units that do not have humidity controls by placing a small, metal pan containing distilled water inside the incubator chamber. Be sure to clean the pan regularly and refill as needed.

Routine Maintenance

Cleaning: The interior of your incubator should be cleaned on a regular basis and when spills occur.

1. Remove contents of incubator and clean walls and shelves with warm water and a phenol-based disinfectant.
2. Rinse interior with clean water and dry thoroughly.
3. Removable parts can be washed in warm sudsy water. Do not use soap-filled or metal scouring pads.
4. Once all parts are cleaned, dried and replaced, verify control settings on unit.

WHEN REPLACING SHELVES AFTER CLEANING,
BE SURE THE SHELVES ARE LEVEL!

Periodic evaluation: As specified in your Operator's Manual, the following components should be regularly inspected as they apply to your incubator:

- ❑ Examine power cord for signs of damage
- ❑ Ensure proper function of door hinges and latches
- ❑ Inspect door gaskets for cracks, distortions, deterioration, and security of attachment
- ❑ Test temperature alarms
- ❑ Check humidity controls
- ❑ Verify proper operation of circulating fans and thermostat

Candle Jars

Many labs use candle jars in conjunction with general-purpose incubators as an economical means of creating a CO₂ environment for the organisms they wish to culture. Glass jars with metal, tight fitting threaded lids are recommended for this purpose. Plastic jars or metal cans with slip-on lids are **not** appropriate because the CO₂ atmosphere is compromised with these types of containers. In addition, only white, unscented candles should be used due to the toxic effects of dyes and perfumes on microorganisms. Remember to allow approximately 3 inches of space at the top when filling the jar to ensure the candle is not extinguished too quickly when lit. Jars should be washed and disinfected weekly.

Lisa Ballance, BS MT(ASCP)

References: Kroll, MH., et al. Laboratory Instrument Evaluation, Verification, and Maintenance Manual, 5th ed., pp. 112-114. College of American Pathologists, Northfield, Illinois. 1999.

Operator's Manual: Thermolyne Type 41900/42000 General Purpose Incubators. Barnstead/Thermolyne Corporation, Dubuque, Iowa. 1992.

Operator's Manual: NAPCO Models 3412 and 3512 Reach In Air Flow Incubators. National Appliance Company, Portland, Oregon.

Instructions: Portable Laboratory Incubator, Model 9333. G.Q.F. Manufacturing Company, Savannah, Georgia.

Operator's Manual: NAPCO Models 5420, 5425, 5440, 5445 Automatic CO₂ Incubators. Precision Scientific, Chicago, Illinois.

Forbes, B., et al. Bailey and Scott's Diagnostic Microbiology, 10th ed., p. 161. Mosby, St. Louis, Missouri. 1998.