INCUBATION OF R-CARD® AND MEMBRANE FILTER MEDIA

The statement “no incubator required” does not mean that no incubation or no incubation time is required. Anytime that a nutrient medium is inoculated with a test sample, a period of time must be allowed before evidence of visible growth is present. That is, the inoculated medium must be incubated.

Anyone with even a rudimentary knowledge of microbiology knows that microbes such as bacteria and molds do not form visible growth or CFU’s (colony forming units or colonies) instantaneously when a sample containing bacteria or mold propagules is added to a nutrient medium. Certain conditions must be met for such growth to occur. These include proper nutrients, moisture and temperature. A proper medium will contain the first two, but temperature is dependent upon where the inoculated medium is incubated. The purpose of an incubator is to provide a constant, set, narrow temperature range that is optimum for the microorganisms you are trying to grow.

Every living organism inherently has a temperature which is optimum for its growth and reproduction. Most organisms have a range within which growth and reproduction will occur which has a lower boundary (the “minimum”) and an upper boundary (the “maximum”), with the “optimum” being somewhere between the two.

An incubator is normally set to maintain temperature close to the optimum for the desired target microorganisms to be grown on the medium. When this is done, the growth of the target organisms is the fastest and the results can be read in the shortest incubation time.

However, when we state that no incubator is needed and instruct that inoculated R-CARD® should be placed in a “warm” area for incubation, we are indicating that the target organisms will grow over a large temperature range. This does not mean that they will grow as quickly as those in the controlled environment of an incubator.

Hence, our instructions are to allow the plates to sit (incubate) in a warm (usually 75-90° F) place and to check them periodically (at 24, 36, 48 hrs.) for visible growth. Once initial growth is seen, we advise giving an additional 24 hrs. time and then doing “final” counts.

Obviously, an incubator is advantageous as it eliminates the temperature variable and allows total reproducibility of that factor. However, for many situations, it is possible to achieve satisfactory results without the use of an incubator. Roth Bioscience is happy to advice on ways to make your own no cost incubator or to direct you to a source where you can buy a very good unit for less than $75.00.

Do I need an incubator and how long should I incubate the Plates?

No, an expensive commercial incubator is not necessary to obtain excellent results. However, conditions do need to be warm enough for the microbes to grow. Therefore, the R-CARD® should be kept in a warm area to allow growth. This could be in a box over a hot air heat register, or better yet in a Styrofoam box where you have placed a bottle of very hot water (when the lid is closed tightly, the heat will diffuse out of the water and warm the inside of the box to a good incubation temperature and hold it for 8-10 hrs., at which point, you can renew the hot water f or further incubation as needed.) Some persons make their own incubators by installing a heating pad, heat tape, or a light bulb inside a Styrofoam or cardboard box, which will hold the temperature steady. However if a light bulb is used, shield the plates so the direct light doesn't hit them and be sure that the bulb is not touching anything that it might ignite. Try to achieve as stable, constant temperature as possible in the area of 85-95° F for most purposes to induce the fastest growth. If you are not using a thermostatically controlled incubator, you should check the plates at intervals, beginning at 24 hrs., until you see some colored (blue E. coli or pink Coliform) spots (colonies) developing (If using ECC medium). Count the colonies if possible and then allow the R-CARDS or MF DISHES to incubate another 24 hrs., and do final counts.

We highly recommend the purchase of a small, inexpensive egg incubator which works extremely well. These can often be found at farm stores for less than $50, and our favorite is made by G.Q.F. manufacturing of Savannah, Georgia. (Phone 912-236-0651, Model # 1602N Hovabator Incubator). These come with an adjustable thermostat and thermometer and we have had one operating in our lab for more than 20 years with no problems. If you are doing ongoing testing, the price is certainly right, and having one of these units eliminates any question of variations from big temperature fluctuations during the incubation period. If you set your incubator at 35 C., you should be able to count results as early as 24 hrs. after placing the plates in the incubator. Do not count any colonies that may develop after 48 hrs. as the E. coli and coliforms that you are monitoring are very fast growing and will definitely be visible within that time period. (For Mold/Yeast media, an incubation temperature of 25-30° C is recommended).