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(54) DEVICE FOR MAKING A CULTURE OF CO₂ REQUIRING ORGANISMS

(71) We, **SMITHKLINE CORPORATION**, of 1500 Spring Garden Street, City of Philadelphia, Commonwealth of Pennsylvania 19101, United States of America, a corporation organized under the laws of the Commonwealth of Pennsylvania, one of the United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to devices for culturing CO₂-requiring micro-organisms.

It is known to culture *Neisseria gonorrhoeae* and other CO₂-requiring organisms in a culture medium retained on an elongate member which is removably positioned in a closed container such as is shown in U.S. Patent Specification No. 3,616,265. It is known to the art to employ a carbon dioxide (CO₂) tablet usually containing sodium bicarbonate and an organic acid, for example citric acid, in the bottom of the container to provide carbon dioxide for culturing the organism. In the presence of moisture, the tablet releases CO₂. Considerable difficulty has been experienced due to the fact that the amount of moisture available to the tablet varies greatly. Frequently, excessive moisture condenses in the container, forming a pool of water surrounding the tablet which causes a rapid evolution of carbon dioxide within minutes producing a higher than usual pressure and a greater than usual leakage through the closure of the container, thereby reducing the length of time that carbon dioxide is present in the container to an unsatisfactory time period.

Devices for culturing micro-organisms are also described in British Patent Specification 1,246,070 and in our copending British Patent Application 13892/76. (Serial No. 1548605).

According to the present invention there

is provided a device for culturing a CO₂-requiring micro-organism, the device comprising an elongate member for supporting the organisms to be cultured, an open ended container for the elongate member and for receiving a culture medium for the organism, a seal for sealing the open end of the container, and securing means for securing a CO₂ tablet to the elongate member between the culture medium and the open end of the container with the tablet exposed to the atmosphere within the container.

Devices of the present invention prevent the tablet from being exposed to an accumulation of water by holding the CO₂ tablet above the culture medium. This results in a substantially even release of carbon dioxide due to the interaction of moisture in the air within the container and the tablet, and it can ensure the presence of carbon dioxide within the container for a period of time sufficient to carry out a desired culturing, for example two days.

Prior art devices and devices in accordance with the invention for culturing a CO₂-requiring organism will now be described, by way of example, with reference to the accompanying diagrammatic drawings in which:—

Figure 1 is a side elevation of a single chamber culture device of the prior art;

Figure 2 is a front elevation of the elongate culture medium-supporting member of the device of Figure 1;

Figure 3 is a side elevation of a two chamber culture device of the prior art;

Figure 4 is a front elevation of the elongate culture medium-supporting member of the device of Figure 3;

Figure 5 is a side elevation of a single chamber culture device in accordance with the invention;

Figure 6 is a front elevation of the elongate culture medium-supporting member of the device of Figure 5;

Figure 7 is a plan view of a carbon dioxide tablet packet;

Figure 8 is a side view of the packet of Figure 7 showing it torn to expose the tablet thereof to the atmosphere;

Figure 9 is a side elevation of a double chamber culture device in accordance with the invention; and

Figure 10 is a front elevation of the elongate culture medium-supporting member of the device of Figure 9.

There is shown in Figure 1 a culture device 1 known to the prior art. The device 1 has a transparent container 2, for example, of, glass or a plastics. An elongate member 4 has an inner end 5 and a recessed portion 6 in each opposed substantially flat face 8. Each recessed portion 6 is intermediate the ends of the elongate member 4 and has multiple small protrusions 10 to facilitate the retention of a body 12 of a solid culture medium in the recessed portions. The elongate member 4 has a handle 16 and a plug portion 18 which is adapted to be sealingly engaged within cylindrical member 20 which has a plug portion 22 received within the open end 24 of container 2. Such a container is disclosed in detail in United States Patent Specification No. 3,616,265. When organisms are being cultured, it is known to introduce into the bottom of container 2 a carbon dioxide tablet 26.

It is also known to employ a double chamber culture device such as that shown at 32 in Figure 3. The device 32 has a transparent container 34 containing an elongate member 36 which supports a culture medium 38 in recesses indicated at 40, 40 in opposite faces 42, 42 of the elongate member 36 and having protrusions 43. Elongate member 36 extends through a ring seal 46 which together with the elongate member 36 separates container 34 into two separate compartments 50 and 52. Chamber 52 contains a liquid culture medium 54. Elongate member 36 has a handle 60 and a plug portion 62 which sealingly engages a cylindrical member 64 which, in turn, has a plug portion 66 which sealingly engages the open end 68 of container 34. Culture devices such as the device 32 have not been employed where it is desired to have carbon dioxide in the upper chamber 50, due to the difficulty of providing a carbon dioxide tablet in chamber 50.

A culture device 70 in accordance with the invention has a transparent container 72 containing an elongate member 74 with recesses 76, 76 in opposite faces 78, 78 containing culture medium 82 and protrusions 83. Elongate member 74 has a handle 86 and a plug portion 88 which sealingly engages a cylindrical member 90

which, in turn, has a plug portion 92 mounted in the open end 94 of container 72. As thus described, the device 70 is the same as the device 1. Elongate member 74 has a slot 100 in which there is secured a packet 102 containing a carbon dioxide tablet 104. Packet 102 can be, for example, opposed sheets 106 and 108 of a plastics such as Cellophane (registered Trade Mark), polyethylene or polypropylene, or a metal foil such as aluminum or tinfoil heat sealed at its edges 110 to be air tight or a combination thereof. Such packets are well known to the art and hence need not be described in greater detail. CO₂ tablets are also well known. Typically the CO₂ tablet 104 will contain a carbonate, for example sodium bicarbonate, and a water-soluble organic acid, for example citric acid or tartaric acid. CO₂ tablets are available commercially, for example, "TAB-CO₂" sold by Ames Company Inc., 1127 Myrtle Street, Elkhart, Indiana, U.S.A. As shown in Figure 7, the packet is activated by punching a pinhole 112 in sheet 106 to provide for the admission of air and moisture within packet 102 to the CO₂ tablet 104 and the exit of the formed carbon dioxide.

As best seen in Figure 6, slot 100 is sized to provide for a wedge fit of the packet 102.

In operation, the sealed packet 102 is punctured to form a pinhole 112, and then the packet is securely wedged in the slot 100. A specimen suspected of including a particular micro-organism is brought into contact with the culture medium 82, and the elongate member 74 is placed in the container 72. If a somewhat faster release of CO₂ is desired, the packet 102 can be provided with a tear 116 adjacent the tablet 104, as illustrated in Figure 8.

Referring now to Figure 9, a two compartment culture device 120 has a transplant container 122 containing an elongate member 124 with recesses 126 in opposite faces 128, 128 containing culture medium 132 and protrusions 129. Elongate member 124 has a handle 136 and a plug portion 138 which sealingly engages a cylindrical member 140 which, in turn, has a plug portion 142 which engages the open end 144 of the container 122. Elongate member 124 passes downwardly through a seal ring 150 which separates the container 122 into an upper compartment 152 and a lower compartment 154 containing a liquid culture medium 156. As thus described the culture device 120 is the same as the culture device 32 shown in Figure 3.

Elongate member 124 has a slot 160 therein which is sized to receive and hold by a wedged fit a packet 102 containing a CO₂ tablet 104.

In operation, the elongate member 124 is

removed from the container 122 while holding the container in a vertical position to retain the liquid culture medium 156 in compartment 154. The container is placed in a holder to maintain it vertical, and the packet 102 is pierced to provide an opening 112 and the packet 102 is then securely wedged in slot 160. The selected specimens are then placed on the lower end of the elongate member 124 for introduction into the liquid culture medium 156 and on the culture medium 126 for culturing in the compartment 152 in the presence of carbon dioxide. Elongate member 124 is then replaced in the container 122. After a suitable period of incubation observations can be made through the transparent walls of the container 122.

A typical solid culture medium for culturing *Neisseria gonorrhoeae* is described by Martin, J. E., Jr. and Lester, A. in HSMHA Health Reports, Volume 86, pages 30—33, 1971.

An example of a CO₂ tablet is a tablet made with a 3/16" punch and having the following composition:

	mg.
Citric Acid (U.S.P. anhydrous granular)	21.320
Sodium Bicarbonate (U.S.P.)	28.000
Terra Alba (U.S.P.)	8.650
Magnesium Stearate (U.S.P.)	0.073

Carbon dioxide within the culturing container is desirable when culturing certain micro-organisms contained in body liquids. Typical of such micro-organisms are *Neisseria gonorrhoeae* and *Streptococci*.

WHAT WE CLAIM IS:—

1. A device for culturing a CO₂-requiring micro-organism, the device comprising an elongate member for supporting the organisms to be cultured, an open-ended container for the elongate member and for receiving a culture medium for the organism, a seal for sealing the open end of the container, and securing means for securing a CO₂ tablet to the elongate member between the culture medium and the open end of the container with the tablet exposed to the atmosphere within the container.

2. A device in accordance with claim 1, in which the securing means comprises an aperture in the elongate member for receiving the CO₂ tablet in a packet.

3. A device in accordance with claim 1 or claim 2, which includes the CO₂ tablet, the tablet containing sodium bicarbonate and citric acid.

4. A device in accordance with any one of claims 1 to 3, which includes the CO₂ tablet which is in a packet having a pinhole opening adjacent the tablet.

5. A device in accordance with any one of claims 1 to 3, which includes the CO₂ tablet which is in a packet having a tear adjacent the tablet.

6. A device for making a culture of a CO₂-requiring micro-organism, the device being substantially as herein described with reference to Figures 5 to 8 or Figures 7 to 10 of the accompanying drawings.

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