

[54] **GAS PERMEABLE STERILE CULTURE BOTTLE**

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[22] Filed: **Apr. 30, 1973**

[21] Appl. No.: **355,754**

[52] U.S. Cl. **195/139, 195/142, 215/1 C**

[51] Int. Cl. **C12b 1/00**

[58] Field of Search **195/139**

[56] **References Cited**
UNITED STATES PATENTS

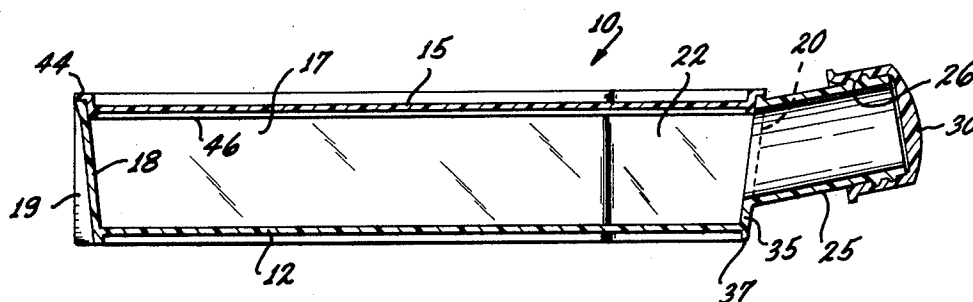
3,301,769	1/1967	Steel	195/139
3,449,210	6/1969	Rohde	195/139
3,726,764	4/1973	White	195/139

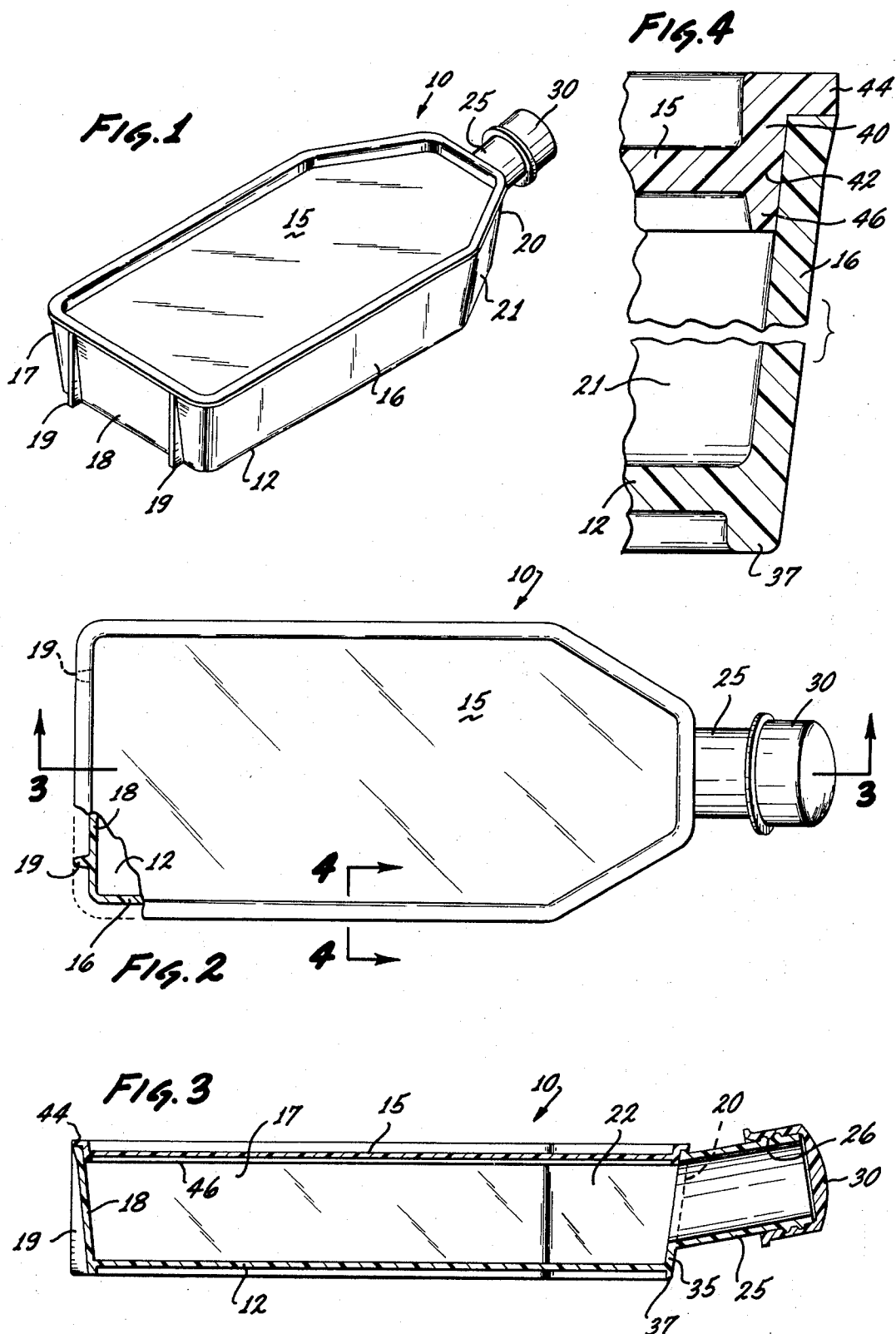
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[57] **ABSTRACT**

A sterile culture bottle in the form of a flat bottomed flask includes a neck opening arranged at an inclined angle with respect to the bottom wall so that a pipette or other instrument may be inserted into the bottle to reach any portion of the contents therein. The side walls, bottom wall and neck are of a unitary integral structure, the top wall being essentially flat and optically transparent and distortion free to permit microscopic examination of the contents within the culture flask. The top wall is of optical quality plastic material, sonically welded to the side walls and hermetically sealed thereto. The top, bottom and side walls of the flask are of an impact resistant plastic which is permeable to gas and water vapor, but impermeable to microorganisms, thus providing better control of the environment within the flask. A typical such plastic is polystyrene having a 2.4 cm. Kg. f/cm. of notch at 23°C (Izod test — ASTM D 256-56). A threaded cap is received over the neck opening to seal the contents of the flask, and the bottom wall is essentially flat to provide stability against tipping of the flask.

10 Claims, 4 Drawing Figures





GAS PERMEABLE STERILE CULTURE BOTTLE

This invention relates to a culture bottle for growing microorganisms, and more particularly to an improved culture bottle which is sterile, permeable to water vapor and gas, but impermeable to microorganisms, the flask being of high impact polystyrene or other suitable plastic and including flat bottom wall, and a spaced flat top wall, the top wall being of optical quality plastic and wherein an inclined neck is integrally formed in the side wall.

Various types of devices are presently employed for growing microorganisms, a typical example of which is the Petri dish. Petri dishes are available in a myriad of types and configurations. Also used are glass tubes or bottles of various dimensions and configurations.

Where the glass bottles or screw-capped test tubes are used for the growth of organisms which require a prolonged incubation period in a CO₂ atmosphere, the practice is to leave the screw caps loose in order to permit passage of the gas into and out of the bottle or test tube. The loose caps operate, however, to enhance dehydration of the culture media. Depending on the looseness of the caps, varying amounts of gas may enter.

Where loose caps are used with glass tubes or bottles, a hazard to the health and safety of laboratory personnel and other employees is present. Breaking the bottle or tipping of the bottle during handling and examination may release droplets containing infectious microorganisms. When such released organisms contact a surface, an aerosol is created and may result in laboratory acquired infections. Such a practice also permits the release of spores and the like. Moreover, the glass tubes and bottles are relatively heavy and when carried in large numbers or in large trays are difficult to handle.

Many times during the incubation of microorganisms, it is the practice to undertake preliminary visual observation or microscopic scanning to determine whether the microorganism is growing, and to attempt some tentative identification of the microorganism. Usually, this is done by removing a small sample from the culture bottle. In the case of pathogenic organisms, this represents a potential hazard. Accordingly, it is desirable to provide a culture bottle in which preliminary visual or microscopic examination may be conducted without removing any of the material from the bottle itself.

U.S. Pat. No. 3,449,210 of June 10, 1969 describes a sterile culture bottle of glass or plastic, including a neck arranged in the side of the flask.

U.S. Pat. No. 3,702,806 of Nov. 14, 1972 describes a disposable triangular shaped culture bottle of transparent plastic.

U.S. Pat. No. 3,532,605 of Oct. 6, 1970 describes a culture bottle in which the culture medium is immobilized by a mesh.

U.S. Pat. No. 2,992,974 of July 18, 1961 describes a glass culture bottle containing a congealed layer of nutrient materials. Petri dishes of polystyrene or other plastic materials are described in U.S. Pat. Nos. 3,097,070 and 2,677,647 of July 9, 1963 and May 4, 1954, respectively.

Other tissue culturing devices and microorganism sampling and culturing units are described in U.S. Pat. Nos. 2,942,520 of June 28, 1960; 3,184,395 of May 18, 1965; 3,203,870 of Aug. 31, 1965; 3,503,665 of Mar. 31, 1970; and 3,696,002 of Oct. 3, 1972.

U.S. Pat. No. 3,346,464 of Oct. 10, 1967 describes a semipermeable plastic envelope for use in a device which is a biological sterility indicator.

U.S. Pat. Nos. 2,920,777 and 3,490,501 of Jan. 12, 1960 and Jan. 20, 1970, respectively, relate to bottles or bottle funnel combinations in which the opening is arranged in the side wall and inclined.

The present invention relates to a much improved disposable sterile culture bottle, which overcomes some of the disadvantages of the prior art devices and practices previously described. In its basic form, the culture bottle of the present invention is in the form of an elongated, flat bottom, flask-like member which includes a bottom wall and a spaced top wall. The side walls are integral with the bottom wall and receive the top wall, which is hermetically sealed thereto. Arranged at one end of the flask is a neck disposed with respect to the bottom wall so as to be at an upwardly inclined angle, the neck having a threaded free end to receive a cap in order to seal the culture bottle to prevent passage of microorganisms therethrough. The portion of the bottle in which the neck is received is tapered slightly so as to avoid sharp right-angle bends rendering the contents of the flask inaccessible to a pipette, or other instrument, inserted through the neck. The top, bottom and side walls and neck of the flask are formed of impact resistant plastic material, which is permeable to gas and water vapor while being impermeable to microorganisms. Moreover, the top wall is of an essentially flat, optical quality transparent plastic arranged essentially parallel to the bottom wall, so as to permit microscopic examination of the culture being grown within the flask.

The sterile disposable culture bottle of the present invention offers the advantage of providing an impact resistant gas permeable flask, which is particularly useful in the incubation of heterotrophic microorganisms in which a gas, such as CO₂, is an essential nutrient. Thus, the gas may permeate through the walls while the entire flask is completely sealed by the cap and while maintaining the proper humidity conditions and gas conditions within the flask itself. From a safety standpoint, the flask is of impact resistant plastic, spillproof because of its design and which includes a tightly stoppered cap. Since the walls are permeable to gas, the cap may be kept tightly on the neck, and accidental spilling during handling of the flask of the present invention is substantially eliminated. Moreover, by providing a top wall which is of optical quality, preliminary visual or microscopic examination of the contents of the flask may be accomplished without the necessity of removing samples from the flask.

Referring to the drawings:

FIG. 1 is a view in perspective of the flask in accordance with the present invention;

FIG. 2 is a plan view, with a portion thereof broken away, of the flask illustrated in FIG. 1;

FIG. 3 is a view in section, taken along the line 3—3 of FIG. 2; and

FIG. 4 is an enlarged fragmentary sectional view taken along the line 4—4 of FIG. 2.

Referring to the drawings which illustrate a preferred form of the present invention, a flask 10 is illustrated in FIGS. 1—3 and includes a flat bottom wall 12 and a spaced top wall 15. Integrally formed with the bottom wall 12 are spaced side walls 16, 17 and 18. Side wall

18 is provided with reinforcing ribs 19 formed integrally therewith, as illustrated.

Positioned oppositely of side wall 18 is a neck wall 20, the latter being joined to side walls 16 and 17 through transition walls 21 and 22, respectively. The transition walls eliminate sharp right-angle bends at the neck end of the flask, and thus eliminate "blind spots" not accessible to a pipette or other laboratory instruments.

Formed integrally with the neck wall 20 is a neck opening 25 threaded, as indicated at 26, to receive a cap 30.

As illustrated in FIG. 3, the neck 25 is arranged at an upwardly inclined angle with respect to the bottom wall 12. In practice, the center axis of the neck 25 is disposed at approximately a 10° angle with respect to the bottom wall 12. In this way, a pipette or other laboratory instrument may be inserted and may easily reach any portion of the interior of the flask. It is for this reason that transition walls 21 and 22 are inclined toward the neck 25 so as to enable access to all portions of the interior of the flask.

As illustrated in FIG. 3, neck wall 20 includes a generally vertical short wall 35 forming the junction between the bottom wall 12 and the neck 25. In normal use, the level of material within the flask is kept below the level of the vertical wall. In the form shown, the neck 25 is closer to the top wall 15 than the bottom wall 12.

As shown, the side walls 16, 17 and 18 and the transition walls 21 and 22 and the neck wall 20 are flared outwardly, such that the overall surface area of the top wall 15 is somewhat greater than the surface area of the bottom wall 12. The surface area of the top wall 15 is about 25 square centimeters, in one form.

The flask 10 is composed of polystyrene plastic which is impact resistant and withstands at least 2.4 cm. Kg. f/cm. of notch at 23°C (Izod test—ASTMD 256-56). This impact resistant characteristic is a desirable safety feature and prevents shattering or breakage of the flask in the event that it is dropped. Overall, the flask 10 has a relatively low center of gravity and a large flat bottom which provides stability against inadvertent tipping.

The flask is permeable to water vapor, oxygen and carbon dioxide and impermeable to any microorganism or spores growing within the flask. The carbon dioxide, oxygen and water permeability of the flask are set forth in the following table in which the data taken was in the absence of a pressure differential:

	at 23°C.	at 37°C.
Carbon dioxide	2.833 ml./24 hrs.	4.166 ml./24 hrs.
Oxygen	1.166	1.166
Water vapor	0.0075 g.	0.0090 g.
(100% R.H. inside bottle, 50% R.H. outside bottle.)		

Referring now to FIG. 4, it will be seen that the bottom wall 12 is formed integrally with the side walls, each of the side walls including an integrally formed leg 37 adapted to rest on a supporting surface, in order to maintain the bottom wall spaced from the supporting surface. In this way, a gas space is created between the underside of the bottom wall and whatever surface the flask is resting on. Accordingly, all wall surfaces are in gas-exchanging relationship with the surrounding environment.

In fabrication of the flask 10, the top wall 15 is formed separately from the remaining structure. The top wall is of a configuration such that its peripheral portion includes a shoulder 40 formed between a peripheral vertical leg section 42 and a horizontal extending lip section 44, the latter overlying the exposed top edge of the mating side walls. The vertical section 42 terminates in a depending foot 46 which is continuous and extends along the periphery of the top wall 15. In this way, there is a substantial surface area between the peripheral portion of the top wall and the mating side walls, such that the two elements may be hermetically sealed together by sonic welding techniques, or other techniques, known to those skilled in the art.

In the formation of the flask 10, which may be by injection molding, and the like, care should be taken to avoid the use of any additives or mold release agents, which can operate as a source of contamination adversely affecting the growth of any microorganism within the flask.

As illustrated in FIG. 3, the top wall 15 is essentially flat, and disposed in parallel relationship with the bottom wall 12. The top wall is preferably of optical quality and transparent, and formed so as to be distortion free. In this way, microscopic examination at 10 power of the contents of the flask 10 through the top wall is possible for purposes of preliminary screening and preliminary visual inspection of the culture being grown within the flask.

The flask above described is particularly useful as a culture bottle for cultures of the *M. tuberculosis* type and other pathogenic or non-pathogenic microorganisms, whose growth depends upon the presence of a gas, such as CO₂ or oxygen or other types of gases. The growth of these microorganisms may be at room temperature or at incubator temperatures, and at temperatures below the boiling temperature of water. The flask of this invention offers the advantage of easy deposition of the microorganism to be grown within the flask, and maintaining the proper gas and relative humidity conditions within the flask while keeping the entire bottle sealed tightly. This is in contrast to the prior practice in which glass bottles were used and the stoppers were loosely positioned over the flask to permit passage of CO₂ to the interior of the flask.

Once the microorganism, spore or fungus to be grown is positioned within the flask of the present invention, the cap may be tightened to prevent entrance of contaminating organisms which may destroy the culture being grown, and more importantly to prevent escape of any culture or microorganisms which could be a source of infection to laboratory personnel and other employees.

After assembly of the flask, as described, it is thereafter sterilized by conventional techniques, e.g., gas sterilization using ethylene oxide. Thereafter, the desired culture medium is introduced into the flask under aseptic conditions, and the flask packaged for shipment. Optionally, the flask may be sterilized and need not contain a culture medium. A typical culture medium which may be employed is, for example, Trypticase Soy Agar, Lowenstein-Jensen Medium, or Agar-Agar with appropriate nutrients. The culture medium is itself well known in the art and any of the known culture media may be employed.

It will be apparent that the flask, above-described, offers singular advantages from the standpoint of safety

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and convenience in the growth of pathogenic and non-pathogenic microorganisms, spores and fungus, i.e., growing biological matter in a nutrient. Safety by providing an impact resistant flask which is relatively stable is another advantage. Due to the gas exchange quality of the walls, the flask may be completely sealed and spillage or accidental release of the contents is substantially eliminated. Preliminary visual or microscopic examination without the necessity of opening and removing a sample is possible. Easy access to all portions of the interior of the flask by the arrangement of the neck and side walls facilitates use and minimizes risk of laboratory infections when the usual degree of care is exercised.

It is obvious that many changes and variations in the design and configuration of the flask and the culture media may be made, as will be apparent to those skilled in the art, without departing from the spirit of the invention as set forth in the appended claims.

We claim:

1. A sterile, disposable culture bottle for growth of microorganisms in a controlled environment of a gas and water vapor comprising: an elongated flask-like member including a bottom wall and a spaced top wall; side walls integral with said bottom wall and defining therewith and with said top wall a flask of unitary structure; one of said side walls including a neck integral with said side arranged at an upwardly inclined angle with respect to said bottom wall, all of said walls and said neck being inseparably secured to each other, said neck having a free end to receive a cap sealably engaging said neck to prevent passage of microorganisms therethrough; said walls of said flask being of an impact resistant plastic material permeable to said gas and water vapor, but impermeable to microorganisms; said top wall being essentially flat, transparent and optically distortion free so as to permit microscopic examination of the culture within said flask, and means on said bottle for maintaining said bottom wall spaced from a supporting surface.

2. A culture bottle as defined in claim 1, wherein said

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bottle contains a solid media for culturing of microorganisms.

3. A culture bottle as defined in claim 1, wherein said plastic is polystyrene.

4. A culture bottle as defined in claim 3, wherein said polystyrene has the following permeability at no pressure differential:

carbon dioxide 2.833 ml/24 hrs. at 23°C and 4.166 ml/24 hrs. at 37°C,

oxygen 1.166 ml/24 hrs. at 23°C and 1.166 ml/24 hrs. at 37°C,

water vapor 0.0075 g/24 hrs. at 23°C and 0.0090 g/24 hrs. at 37°C,

said water vapor permeability being measured at 100% R.H. within the bottle and 50% R.H. outside of said bottle.

5. A culture bottle as defined in claim 1, wherein said plastic has an Izod impact of 2.4 cm. Kg. f/cm. of notch at 23°C as determined by ASTM D 256-56.

6. A sterile culture bottle as defined in claim 1; wherein said neck is arranged at an angle of about 10° with respect to said bottom wall.

7. A sterile culture bottle as defined in claim 1, wherein said bottom wall is essentially flat, and said top wall being in essentially spaced parallel relative to said bottom wall thereby permitting microscopic examination through said top wall of the culture being grown with said flask.

8. A sterile culture bottle as set forth in claim 5, wherein said top wall is essentially flat and of the same plastic material as the remainder of said flask-like member.

9. A sterile culture bottle as set forth in claim 1, wherein said side walls include transition walls adjacent said one wall which includes said neck thereby eliminating right-angle walls adjacent to said wall which includes said neck.

10. A sterile culture bottle as defined in claim 1, said means comprising legs adapted to rest on the supporting surface.

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