Oct. 6, 1970 A. BLOCH ET AL 3,532,604

BIOLOGICAL PACKAGE

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FIG. 1

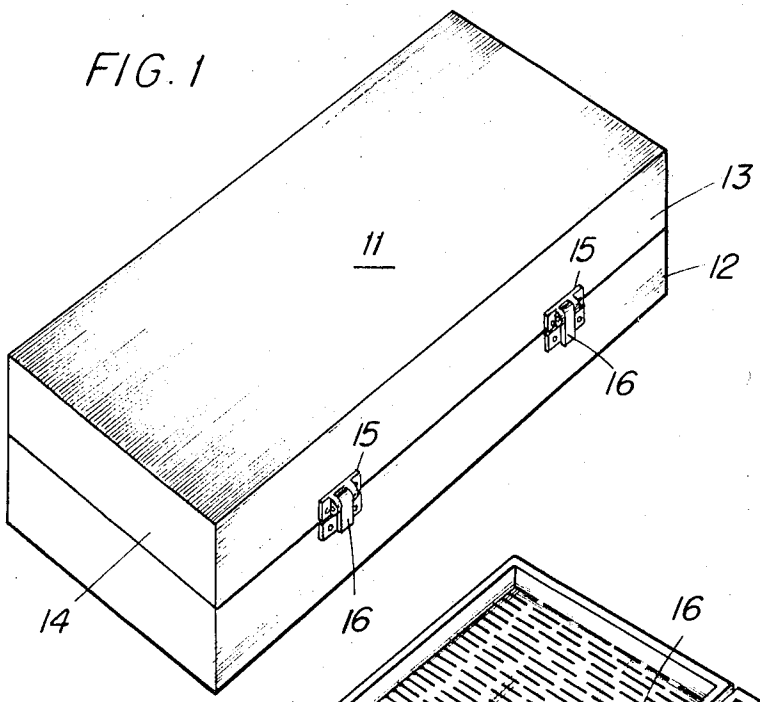


FIG. 2

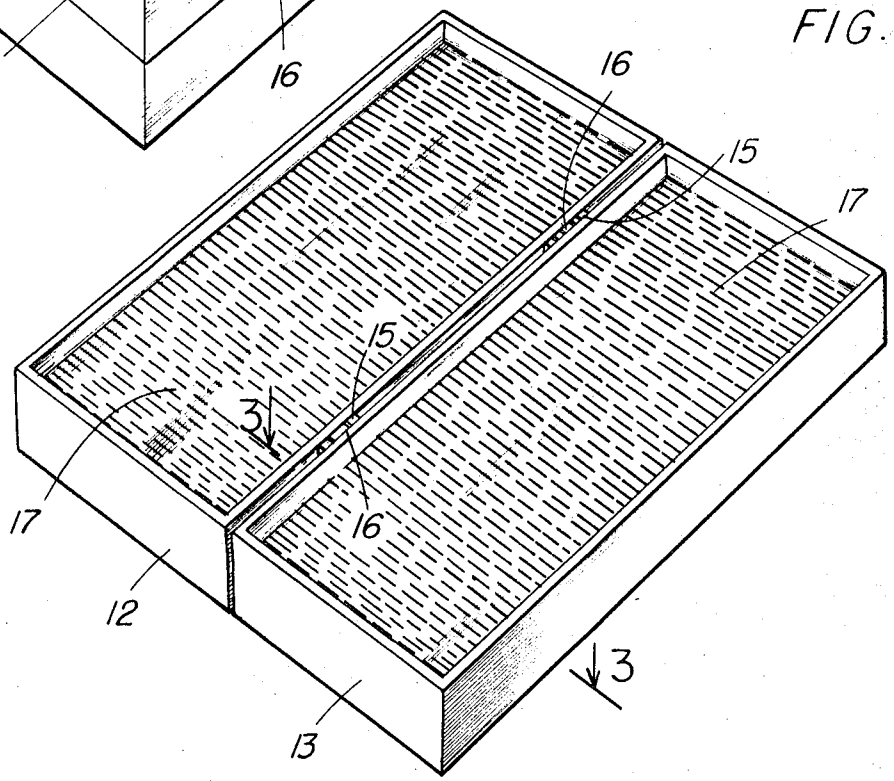
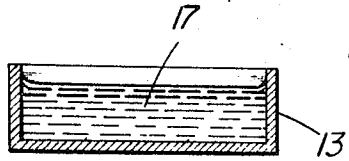


FIG. 3



Inventors
Alfred Bloch
Eugene H. Bernstein

By

Robert D. Flynn

ATTORNEY

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Filed Oct. 3, 1967 4 Sheets-Sheet 2

FIG. 4

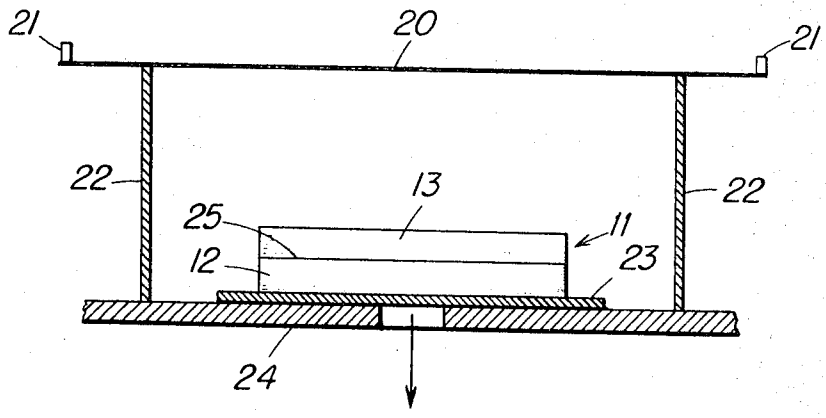


FIG. 5

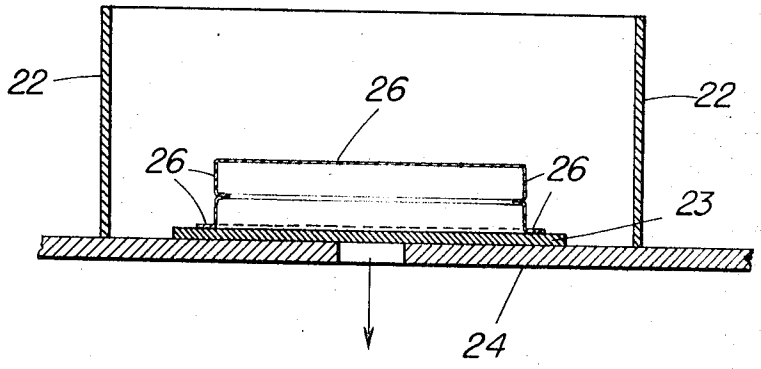
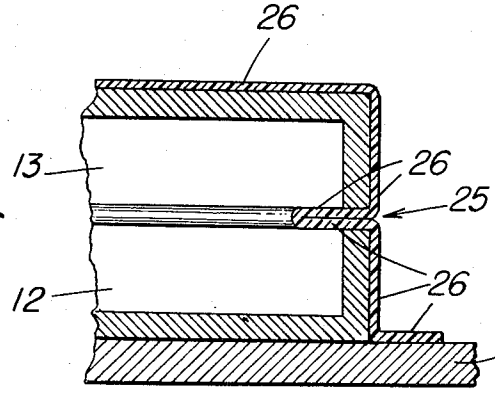


FIG. 6



Inventors
Alfred Bloch
Eugene H. Bernstein
By
Robert D. Flynn
ATTORNEY

Oct. 6, 1970 A. BLOCH ET AL 3,532,604

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Filed Oct. 3, 1967 4 Sheets-Sheet 3

FIG. 7

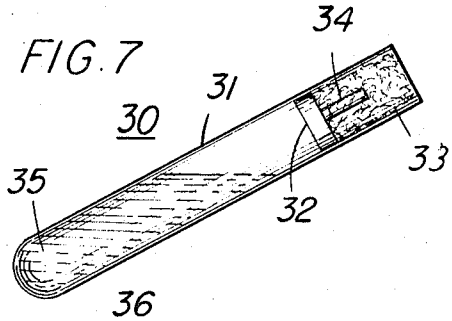


FIG. 8

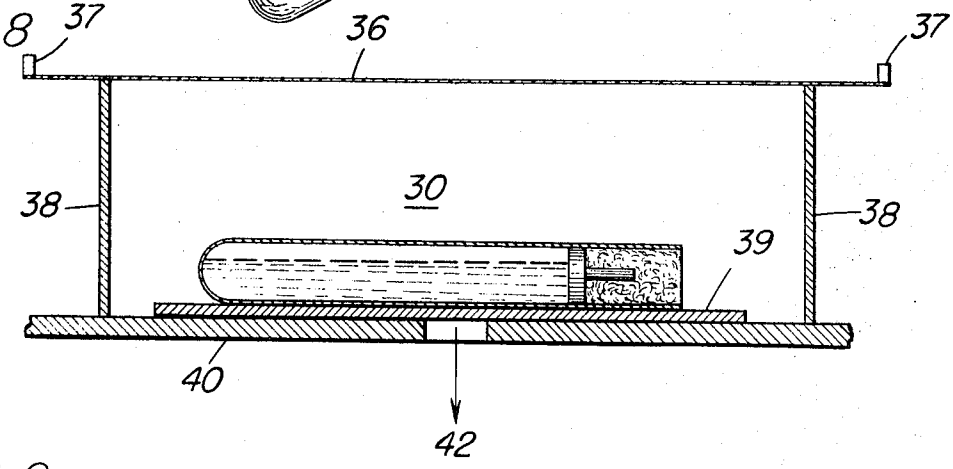
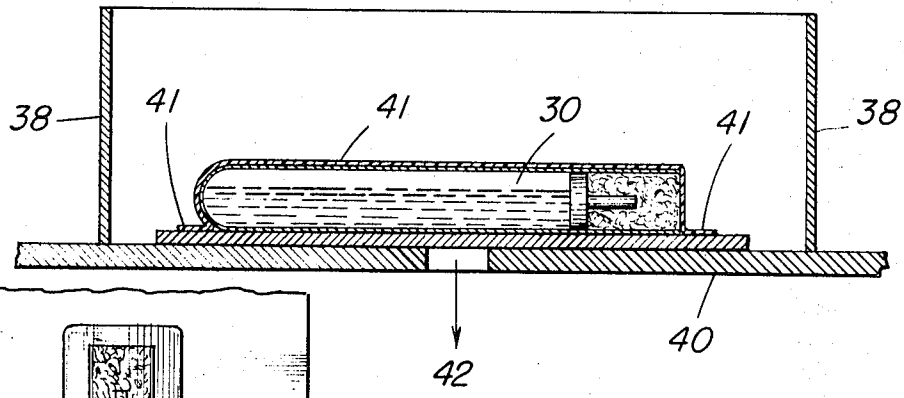


FIG. 9



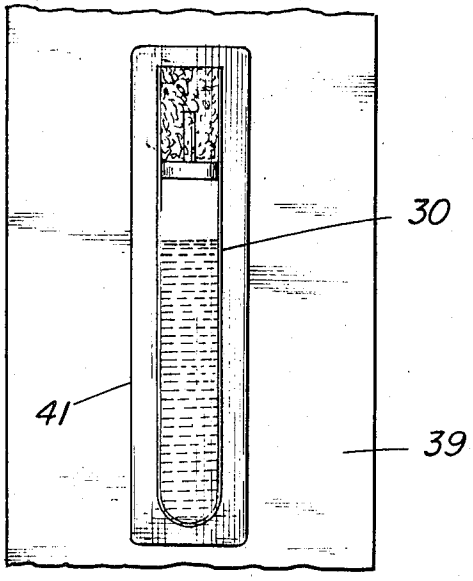


FIG. 10

Inventors
Alfred Bloch
Eugene H. Bernstein

By

Robert D. Flynn
ATTORNEY

Oct. 6, 1970 A. BLOCH ET AL 3,532,604

BIOLOGICAL PACKAGE

Filed Oct. 3, 1967 4 Sheets-Sheet 4

FIG. 11

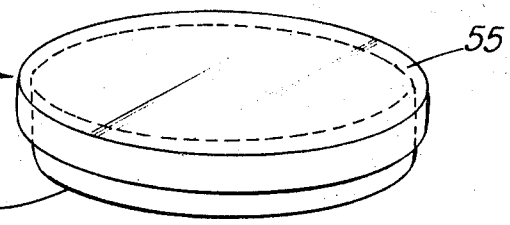


FIG. 12

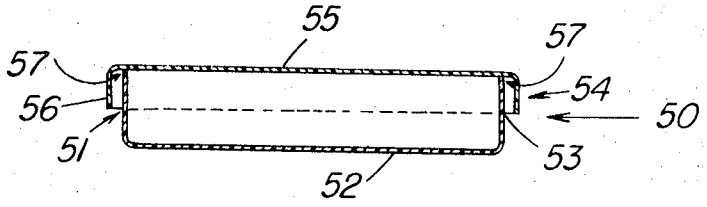


FIG. 13

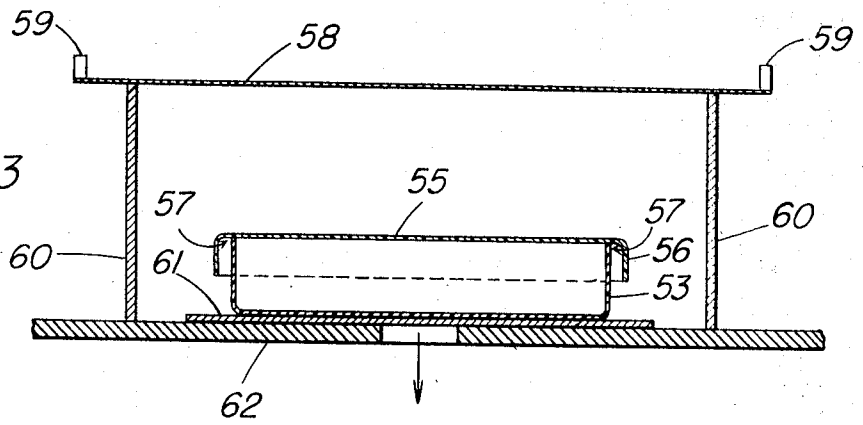


FIG. 14

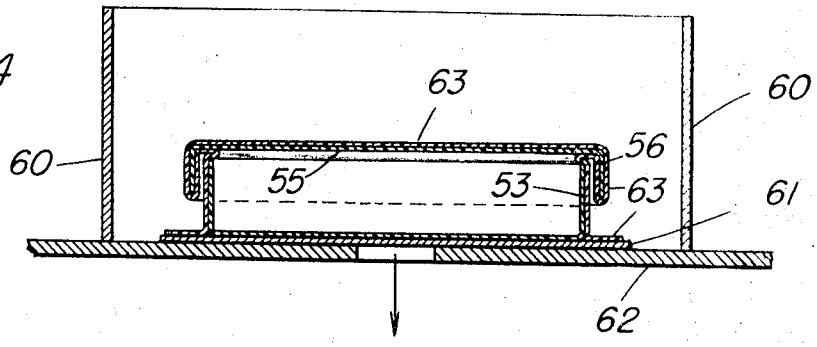
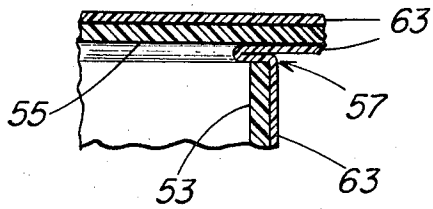


FIG. 15



Inventors
Alfred Bloch
Eugene H. Bernstein

By

Robert D. Flynn
Attorney

United States Patent Office 3,532,604 Patented Oct. 6, 1970

3,532,604

BIOLOGICAL PACKAGE

Alfred Bloch, 9 Bloomfield Ave., Somerset, N.J. 08873, and Eugene H. Bernstein, 901 S. Park Ave., Highland Park, N.J. 08904

Filed Oct. 3, 1967, Ser. No. 672,636
Int. Cl. C12k 1/10
U.S. Cl. 195—139 6 Claims

ABSTRACT OF THE DISCLOSURE

Biological package in which a biological material is contained, is protected against air and microorganisms and its moisture content is retained therein. The package includes an essentially non-porous container having a body member and a cover member fitting together in a substantally hermetically sealed relationship. A pliable film of an ionic copolymer fills the aperture defined by joining of the body and cover members, or can adhere to the exterior of the container.

This invention has to do with a biological package and particularly with a package in which a biological material is protected against deteriorative oxidation, dehydration and against microorganisms.

Problems have existed for many years, and exist today, in regard to the storage, handling, transportation and preservation of biological materials. This is particularly pronounced with microbial culture media dispensed in containers used for growing microbes. Culture media are relatively sensitive compositions; they are particularly prone to deteriorate on exposure to air and when contaminated by microorganisms. In particular, when water is generally associated with the media evaporates therefrom, thus lowering the water content of the culture media, the support of growth is reduced or can be fully inhibited. In the case of the conventional Petri dish culturing device, the bottom section containing a culture medium and a lid therefor can disengage during storage, handling or transport; as a result, air or microorganisms can come into contact with the medium and contaminate it, and moisture from the culture medium can evaporate therefrom. With such a device as a Petri dish, expensive means must be adopted to prevent the bottom section and lid from disengaging during transport.

Disposable culture devices have also been developed in an effort to overcome problems encountered with the conventional Petri dish. One such device comprises a Petri dish housed in a plastic envelope. Complete disengagement of the bottom section and the lid is prevented by the confining action of the plastic envelope. However, motion between the bottom section and lid occurs, such that moisture escapes from the culture medium and the Petri dish. The moisture accumulates within the plastic envelope but outside the Petri dish. Here again, then the ability of the medium to support growth is reduced. Also, the moisture so accumulating outside the dish can cause microorganisms to spread. Moreover, the moisture accumulating within the plastic envelope is unsightly, seriously reducing the aesthetic value of the package. It will be apparent that the sterility of the inner surface of the plastic envelope is important and that the envelope offers little or no protection to the Petri dish during transportation.

Another development in modifying Petri dishes involves the use of a plastic Petri dish the two members of which are locked together mechanically and sealed with a composition of wax-like consistency. Yet, the seal has been inadequate since severe dehydration has been observed visually. Microbial contamination has been found to occur

in less than six weeks storage with Trypticase-Soy Agar culture medium and with Trypticase-Soy Blood Agar culture medium. When the same media were contained in such devices and the devices were aged in a refrigerator at 0–4° C., water droplets formed on the outside cover member of the Petri dish after 6 weeks by condensation of water which had escaped from the media. Growth was satisfactory in both media, after inocculation with pathogenic *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Salmonella schottmuelleri*. After twelve weeks in the refrigerator at 0–4° C., both culture media looked to be partially dehydrated, droplets of water which had escaped from the media condensed outside of the Petri dish covers and the Trypticase-Soy Blood Agar had darkened slightly. Growth of the several microorganisms mentioned above, was supported poorly and the Trypticase-Soy Blood Agar culture medium split during incubation, thereby indicating advanced dehydration. Thus, the shelf life of the locked and sealed Petri dishes is less than six weeks at room temperature (20–25° C.) and is about twelve weeks at about 0–4° C.

Still other efforts have been made to preserve, store and transport biological materials. Screw top bottles and tubes have been used, but the seal between the body member and cover member has invariably been incomplete. Sealed glass ampoules have been used; but with the recognition of several disadvantages, including high cost, glass breakage, dangerous equipment and shipping problems. Plastic form-fitting seals, such as those used on chemical and reagent bottles, have been employed. However, the seals become brittle with time and do not adhere to all surfaces.

Films such as waxed paper, aluminum foil, polyethylene and Saran (vinylidene chloride) have been tried. Such films do not adhere to a variety of surfaces with the result that moisture losses and gas exchange occurs. The opportunity for contamination prevails.

Other developments involve change of the physical and/or chemical state of the biological material, by such procedures as freeze-drying (lyophilization), deep freezing and the addition of preservative chemicals. All of these approaches change the nature of the biological material, often to their detriment. In addition, expensive equipment is required.

The invention is illustrated hereinafter with reference to the accompanying drawings in which:

FIG. 1 is a perspective view of a typical container;

FIG. 2 is another perspective view of the container of FIG. 1 opened and with a culture medium in the body member and in the cover member;

FIG. 3 is an elevation cross-section of one of the members of FIG. 2 along 3—3 of FIG. 2;

FIG. 4 is a schematic side-elevation view of the container of FIG. 1 closed and in position to be covered with a film;

FIG. 5 is a schematic side-elevation view of the container of FIG. 4 covered with the film;

FIG. 6 is an exaggerated sectional view of one-end of the container of FIG. 1 covered with the film;

FIG. 7 is a side view of another typical container, a tube;

FIG. 8 is a schematic side-elevation view of the container of FIG. 7 in position to be coverd with a film;

FIG. 9 is a schematic side-elevation view of the container of FIG. 7 covered with the film;

FIG. 10 is a schematic vertical side-elevation view of the container of FIG. 7 covered with the film and secured to a slightly porous member;

FIG. 11 is a perspective view of a Petri dish and cover;

FIG. 12 is a side view of the container of FIG. 11;

FIG. 13 is a schematic side view of the container of FIG. 11 in position to be covered with a film;

FIG. 14 is a schematic side view of the container of FIG. 11 covered with the film;

FIG. 15 is an exaggerated fragmentary cross-section of one end of the container of FIG. 11 covered with the film.

In accordance with the present invention, there is provided a biological package comprising

(a) An essentially non-porous container having a body member and a cover member therefor, said body and cover members fitting together in a substantially hermetically sealed relationship,

(b) A biological material susceptible to deterioration on exposure to air and to microorganisms, within said container, and

(c) An impermeable, pliable film having a thickness of from about 5 to about 10 mils adhering to and filling the aperture defined by joining of said body and cover members, the film comprising an ionic copolymer of an alpha olefin having the formula R—CH=CH_2, where R is hydrogen or an alkyl group having from 1 to 8 carbon atoms, and an alpha, beta-ethylenically unsaturated carboxylic acid having from 3 to 8 carbon atoms, the copolymers having from 10% to 90% of the carboxylic acid groups ionized by neutralization with metal ions uniformly distributed throughout the copolymer.

Another embodiment of the invention is a corresponding package in which the film adheres to substantially the entire exterior surface of the container.

Thus, an essential feature involves an impermeable, pliable film closing the aperture formed by joining of the body and cover members.

Materials useful for the container can be any material which can be shaped, has sufficient strength to withstand use and transportation, and satisfies bacteriological requirements. With regard to bacteriological requirements, a material must be sterilizable and must not interfere with microorganism growth. Particularly desirable are synthetic polymeric materials, plastics, including polystyrene, polyvinylchloride, polyethylene, polypropylene, methylmethacrylate, cellulose acetate, and nylon (a polyamide resin made by polymerization of the hexamethylene diamine salt of adipic acid.) Advantages possessed by such synthetic polymers are their capacity to be molded to accurate dimensions, light weight and resistance to fracture (in contrast to glass). Transparent plastic containers are advantageous since the culture medium contained therein can be readily analyzed microscopically in place. However, glass can also be used.

Biological media of a wide variety can be used in the packages of this invention. For example, culture media include: Trypticase Soy Agar; Trypticase Soy Blood Agar; Eugon Agar; Nutrient Agar; Antibiotic Base and Seed Agars; Brilliant Green Agar; Chapman-Stone Medium; Staphylococcuc 110 Medium; Desoxycholate Agar; Eosin Methylene Blue Agar; Salmonella-Shigella Agar; and Sabouraud Agar.

Still other biological materials contemplated herein include: mammalian cells, organs and tissues; blood, blood cells and blood fractions; body exudates, secretions, extracts and semen; tumor and transplant cells, tumor and transplant tissues, tumor and transplant extracts, corresponding biological components of other vertebrates and from other invertebrates; botanical seeds, grains, cells and parts for grafting; roots, fruits, vegetables, flowers, nuts, meats, vegetables, and other foodstuffs; bacteria, fungi, rickettsia and viruses; growth media, growing colonies and cells; tissue culture cells and media; and enzymes.

As indicated an impermeable, pliable film is an integral portion of the package. The film has a thickness of from about 5 to about 10 mils. Lesser thicknesses are disadvantageous in that structural strength is reduced substantially and impermeability suffers; greater thicknesses are to be avoided because of a reduction in pliability and adaptibility; additionally, vacuum or pressure sealing becomes more difficult, and expense increases.

The film comprises an ionic copolymer of an alpha olefin having the formula R—CH=CH_2, where R is hydrogen or a C_1–C_8 alkyl group, and an alpha, beta-ethylenically unsaturated carboxylic acid having from 3 to 8 carbon atoms, the copolymer having from 10% to 90% of the carboxylic acid groups ionized by neutralization with metal ions uniformly distributed throughout the copolymer. The olefin content of the copolymer is at least 50 mol percent of the copolymer, and the acid monomer content of the copolymer is from 0.2 to 25 mol percent based on the copolymer. The acid can have 1 or 2 carboxylic acid groups.

Typical α-olefins employed in the copolymer include ethylene, propylene and butene-1. Representative alpha, beta-ethylenically unsaturated carboxylic acids include acrylic acid, methacrylic acid, maleic acid and maleic anhydride (which can be considered an acid herein since its chemical reactivity is similar to that of maleic acid).

A preferred copolymer is one formed of ethylene and methacrylic acid, marketed by Du Pont under the designation "Surlyn."

The film adheres to a wide variety of surfaces including glass, plastic, paper products, wood, metal, asbestos and containers of any size or shape. Transparency of the film permits observation of the state of the biological medium at all times. The film has a low water vapor transmission rate and a low oxygen permeation rate.

Further details in the preparation and composition of the film component of this invention are available in U.S. Pat. No. 3,264,272 of Rees.

Referring more specifically to FIG. 1, numeral **11** generally designates a container body which includes body member **12** and cover member **13**. Side wall **14** is shown with hinge members **15** and **16** which join or mate to from a hinge unit. The hinging mechanism is illustrative only and body and cover members **12** and **13**, respectively, can be joined together by any hinging mechanism serving to provide a tight-fitting closure. Body and cover members **12** and **13**, respectively, can serve the same function by containing culture medium. Typical dimensions are from 1″ x 1″ x ¼″ to 5″ x 5″ x 1″, with the depth being from ¼″ to 1″.

FIG. 2 depicts container body **11** opened fully, body member **12** and cover member **13** being joined by the hinging mechanisms formed by members **15** and **16**. Culture medium is indicated by numeral **17**.

As shown in FIG. 3, culture medium is contained in cover member **13** just as it can be in body member **12**. Each member is usually not completely filled; frequently, each is filled to about one half of capacity, the remainder being air space.

In FIG. 4, container **11** is shown in position to be covered with a suitable film of ionic copolymer **20**, which is held in place by clamps **21** and which is supported by supporting members **22**. Container **11** is positioned upon a perforated or slightly porous member **23**, such as cardboard, which, in turn, is supported upon base members **24**. The juncture of body member **12** and cover member **13** is identified as **25**.

In FIGS. 5 and 6 container **11** is shown with the film **20** forming a skin **26** thereon. This skin or film is formed on container **11** by applying a vacuum to the assembly shown in FIG. 4. A vacuum beneath film **20** in FIG. 4 causes atmospheric pressure to push the film down onto container **11** and porous member **23**. As the film **20** contacts container **11**, it forms a continuous, adherent skin to container **11** and porous member **23**. The film fills whatever open space exists at the junction **25** of the meeting of body member **12** and cover member **13**. If desired, the bottom surface of porous member **23** (that is, the surface thereof not in contact with container **11**) can be made less permeable or even impermeable by a subsequent treatment. For example, that surface can be

sealed with a suitable sealant such as collodion, a varnish or resin.

FIG. 7 depicts an example of packaging a liquid biological medium. Tube container **30** has tube **31** serving as the body member and stopper disc **32** or other suitable closure serving to contain liquid biological medium indicated by **35**. Plastic foam or cotton plug **33** serves as a bacteriological closure where required. Stopper disc **32** has perpendicular projection **34** to aid in the removal of the disc from tube **31**. Liquid growth media in tubes generally require air. Disc **32** prevents wetting of the foam or cotton by the liquid media, and also prevents air from reaching the liquid until desired. Removal of plug **33** and disc **32** from tube **31**, followed by replacement of plug **33** provides a means of retaining a sterile system with desired gas exchange.

FIG. 8 reveals tube **30** in position to be covered with film **36**, the film being held in place by clamps **37** and supports **38**. Tube **30** is positioned upon slightly porous member (e.g., cardboard) **39**, which, in turn, is supported upon base members **40**.

FIG. 9 shows tube **30** with the film **36** forming a skin **41** thereon. Heat is applied by a heating element (not shown) in order to soften the film. This skin or film is formed on tube **30** by applying a vacuum to the assembly shown in FIG. 8. A vacuum beneath film **36** in FIG. 8 causes atmospheric pressure to push the film down onto and forms a continuous, adherent skin onto tube **30** and member **39**. As the vacuum is applied, air is removed through passage **42** between base members **40** which are spaced apart to allow for air removal.

FIG. 10 shows container **30** secured to member **39**, with the film or skin **41** adhering to both.

As indicated with FIGS. 7–10, bacterial growth medium or other biological material in the liquid state can be prepared, skin-wrapped, transported and enjoy the same degree of protection from moisture loss and gas exchange as the solid materials illustrated with FIGS. 1–6. Thus, tube **30** can be sterilized before or after skin-wrapping. It has been found that irradiation does not reduce the adherent characteristics of Surlyn, nor does storage at —20° C. or at 40° C. In use, the tube **30** is removed from the skin-wrap **41**, the plug **33** is removed and the perpendicular projection **34** engaged for removal of the stopper **32**, if desired. The plug **33** is then replaced as indicated above, and the tube **30** is available as a test medium.

In FIGS. 11–15, there is shown a Petri dish **50** having a relatively shallow cylindrical configuration. Dish **50** includes body member **51** comprising a base **52** and depending side wall **53**. Dish **50** also includes cover **54** which is of substantially the same shape as body member **51** and which is somewhat more shallow and possesses a substantially greater diameter so that it can fit loosely over the open upper end of **51**. Cover **54** comprises top **55** with depending side wall **56**. The juncture of body member **51** and cover **54** is identified as **57**.

As shown in FIG. 13, dish **50** is positioned to be covered with film **58**, which is held in place by clamps **59** and supported by supports **60**. Dish **50** is positioned upon slightly porous member **61** which, in turn, is supported upon base members **62**.

In FIG. 14, dish **50** is shown with the film **58** forming a skin **63** thereon. The skin is formed in the same manner as described above in connection with FIGS. 5 and 6. The film fills whatever open space exists at the junction **57** of the meeting of body member **51** and cover **54**, as shown in FIG. 15.

Methods for applying a film to a substrate to form a skin or impervious covering thereon, are well known in the art. Thermoforming or vacuum forming techniques are typical. Details are given, for example, in Modern Plastics Encyclopedia, vol. 44, pp. 736–745, 1967.

The invention is illustrated further by the following typical examples.

Test organisms used were strains of pathogenic and non-pathogenic organisms, including: *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Salmonella schottmuelleri.*

The culture media used were Trypticase-Soy Blood Agar, Trypticase-Soy Agar, Eugon Agar and Staphylococcus Medium #110.

EXAMPLE 1

Hinged plastic boxes of the type shown by FIGS. 1–3 were used. The body members **12** and cover members **13** were partially filled with Eugon Agar. The boxes were closed and then covered by Surlyn of 5 mil thickness by following the sealing procedures described and illustrated by FIGS. 4–6 above. The boxes were then sterilized by irradiation with cobalt-60.

Several of the boxes were aged at room temperature (20–25° C.) for 27 weeks. The culture media appeared to be somewhat thinner but supported growth of each of the test organisms identified above. After aging for 27 weeks under refrigeration (0–4° C.), the appearance of the culture media was excellent and growth of each of said organisms was excellent.

EXAMPLE 2

Following the procedure of Example 1, Staphylococcus Medium #110 was used in place of Eugon Agar. This culture medium aged for 27 weeks at room temperature (20–25° C.) or aged under refrigeration (0–4° C.), supported growth and differentiated pathogenic *S. aureus* from non-pathogenic *S. aureus.*

EXAMPLE 3

Commercially available, conventional plastic Petri dishes were employed. Trypticase-Soy Blood Agar was added to the bottom section thereof and the lid was placed over that section. Each Petri dish was wrapped with Surlyn (5 mil thickness) by following the procedure described above in connection with FIGS. 13–15. After an aging period of 6 weeks at room temperature (20–25° C.), the culture medium was still bright red.

In other Petri dishes, the culture medium employed was either Trypticase-Soy Agar, Eugon or Staphylococcus Medium #110. After a similar period, the other culture media appeared to be unchanged. No fluid or condensate could be observed between the Petri dish and the film. There was no contamination. All media supported growth excellently with each of the test organisms mentioned above.

After an aging period of 12 weeks at room temperature (20–25° C.), the Trypticase-Soy Blood Agar had a dark brown discoloration, had become thin and did not support growth. The Trypticase-Soy Agar had become thinner also, but gave a fair growth with all test organisms mentioned above. Eugon and Staphylococcus Agar were in good condition and supported growth well.

After an aging period of 12 weeks under refrigeration at 0–4° C., all culture media looked unchanged and gave excellent growth of all test organisms mentioned above.

EXAMPLE 4

The procedure of Example 1 was repeated with the following modifications. A group of containers was covered with Surlyn by following the procedure given above in connection with FIGS. 4–6. Surlyn film covering the containers was removed except that a band of film was left intact about the junction of body member **12** and cover member **13** extending continuously about the container. Each container was placed in a separate polyethylene bag which was then sealed. Each bag was stored in a refrigerator, at 0–4° C., for 4 months. After 4 months, the bags holding the containers showed no moisture.

Sealing of the aperture **25** with a tape such that the tape adequately fills and seals the aperture is a difficult operation and, in practice, is generally unsuccessful.

Thus, for example, when a variety of tapes were used to seal aperture **25**, the seal proved to be inadequate as evidenced by substantial moisture loss from container **11**. This is illustrated in Comparative Example A below.

COMPARATIVE EXAMPLE A

Collodion, plastic solvent and a variety of pressure sensitive tapes were applied individually to body member **12** and cover member **13** of each of a group of containers **11** partially filled with Eugon Agar, in the immediate area of the junction thereof forming aperture **25**. When the containers were stored at room temperature and at 0–4° C., moisture migrated from the culture media and escaped from the containers.

COMPARATIVE EXAMPLE B

When a variety of other films were applied to and covered a container such as used in Example 1, it was found that moisture migrated from the container and, in some instances, microbial contamination occurred in the container. Film such as the following were found to be unsatisfactory: Saran (despite its negligible water vapor transmission rate and negligible oxygen permeability rate), polyethylene, collodion, aluminum foil, tin foil, aluminum-plastic foils and plastic tapes.

Although the present invention has been described and illustrated with reference to specific illustrations, it is to be understood that modifications and variations of the several elements of the culture devices may be made by those skilled in the art within the principle and scope of the invention as expressed in the appended claims.

We claim:

1. A biological package comprising:

(a) an essentially non-porous container having a body member and a cover member thereof, said body and cover members fitting together in a substantially hermetically sealed relationship,

(b) a biological material susceptible to deterioration on exposure to air or by loss of moisture and to microorganisms, within said container, and

(c) an impermeable, pliable film having a thickness of from about 5 to about 10 mils adhering to as a skin and filling the aperture defined by joining of said body and cover members, the film comprising an ionic copolymer of an alpha olefin having the formula $R—CH=CH_2$, where R is hydrogen or an alkyl group having from 1 to 8 carbon atoms, and an alpha, beta-ethylenically unsaturated carboxylic acid having from 3 to 8 carbon atoms, the copolymers having from 10% to 90% of the carboxylic acid groups ionized by neutralization with metal ions uniformly distributed throughout the copolymer.

2. A package defined by claim **1** wherein the film adheres to a major portion of the entire exterior surface of the container.

3. A package defined by claim **1** wherein the biological material of (b) comprises a solid, a gel-like or a liquid culture supporting medium.

4. Apackage defined by claim **3** wherein a gel-like culture supporting medium is secured to the body member interior and to the cover member interior.

5. A package defined by claim **1** wherein container (a) is sterile and material (b) is aseptically incorporated therein.

6. A package defined by claim **1** wherein the film of (c) comprises a copolymer of ethylene and methacrylic acid.

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ALVIN E. TANENHOLTZ, Primary Examiner

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