March 16, 1954 A. GOETZ 2,672,431

MEANS FOR PERFORMING MICROBIOLOGICAL ASSAYS OF AEROSOLS AND HYDROSOLS

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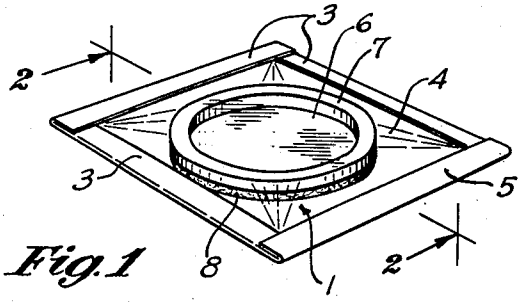


Fig. 1

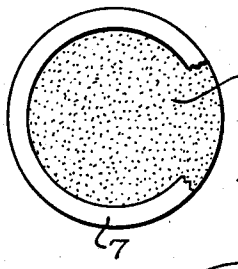


Fig. 3

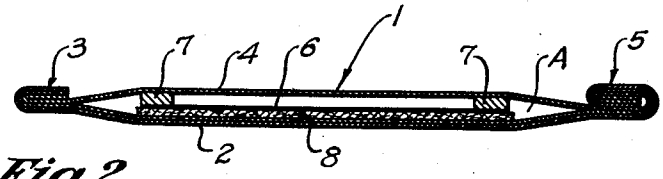


Fig. 2

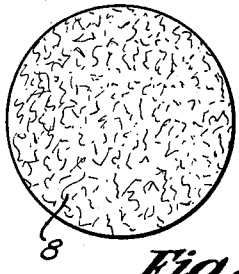


Fig. 4

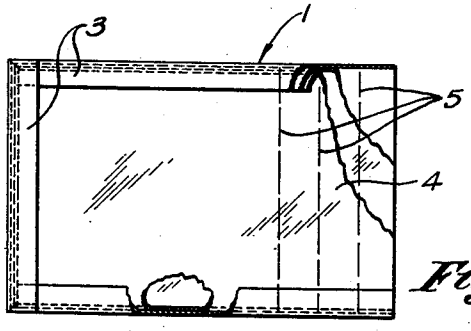


Fig. 5

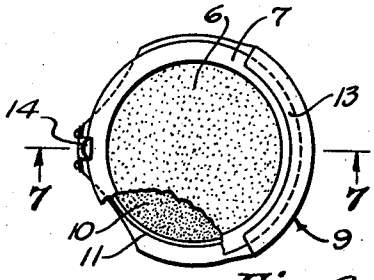


Fig. 6

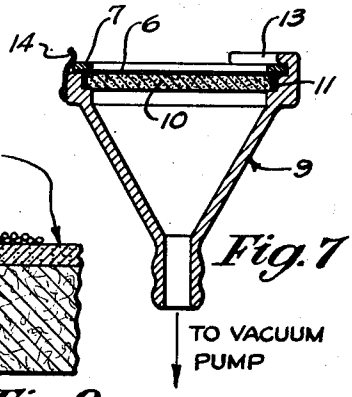


Fig. 7

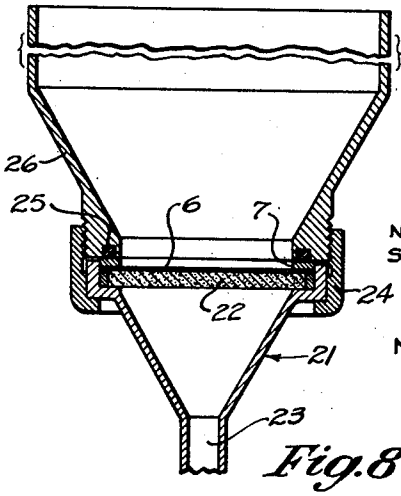


Fig. 8

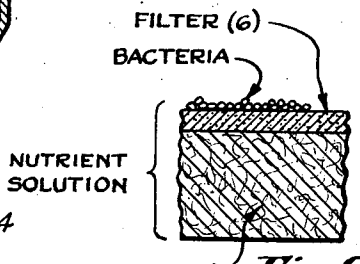


Fig. 9

TO VACUUM PUMP

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MEANS FOR PERFORMING MICROBIOLOGICAL ASSAYS OF AEROSOLS AND HYDROSOLS

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7 Claims. (Cl. 195—139)

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My invention relates to microbiological assaying; more particularly to means and method of performing microbiological assays of either aerosols or hydrosols. Included in the objects of my invention are:

First, to provide a means and method of microbiological assaying which involves simple and compact equipment so arranged as to eliminate the need of aseptic handling and thus especially suited for use under field conditions or under adverse conditions and by operators having a minimum experience.

Second, to provide a means and method of microbiological assaying wherein the equipment may be stored ready for use at any time without delay for such previously required operations as sterilization, preparation of nutrient, and coating of the nutrient substrate.

Third, to provide a means and method of microbiological assaying wherein the time required to prepare for, collect and seal each sample is reduced to a minimum, whereby an assay may be performed upon minimum notice and in a minimum of time to meet conditions of emergency.

Fourth, to provide a means and method of microbiological assaying wherein the collecting units or microfilters themselves become the permanent record thus greatly facilitating the maintenance of such records and permtting the counting and analysis of the developed growth at later times, as well as immediately after the incubation period is over.

With the above and other objects in view as may appear hereinafter, reference is directed to the accompanying drawings in which:

Figure 1 is a perspective view of the assaying unit or cell forming a part of my invention.

Figure 2 is a longitudinal sectional view thereof taken through 2—2 of Figure 1.

Figure 3 is a plan view of a filter membrane.

Figure 4 is a similar view of the nutrient pad.

Figure 5 is a plan view of the pocket member which receives the filter membrane and the nutrient pad.

Figure 6 is an end view of a microfilter holder suitable for the assaying of aerosols.

Figure 7 is a sectional view thereof through 7—7 of Figure 6.

Figure 8 is a sectional view of a holder suitable for the assaying of hydrosols.

Figure 9 is a greatly enlarged or exaggerated fragmentary view of the microfilter and pad after exposure to illustrate the manner in which the nutrient is supplied to the bacteria.

For the purpose of my invention, I employ an assaying assembly which includes a pocket structure 1. The pocket structure comprises a sheet 2 of metal foil, three margins of which are folded as indicated by 3 and sealed to the margins of a transparent window member 4. The window member is formed of a flexible, impermeable plastic film such as rubber hydrochloride known to the trade as "Pliofilm," or a wax coated viscose, known commercially as "cellophane MSAT type" which is moistureproof and heat sealable. The window member is preferably folded to form a double wall defining an outer pocket A between the two layers, the inner layer forming a protective covering over the foil sheet 2.

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One end of the pocket A thus formed is open, but may be closed by folding the metal foil and window as indicated by 5 in Figures 1, 2, and 5. The foil is preferably aluminum. If desired, the extra layer of the window may be omitted in which the inner side of the foil is lacquered to prevent growth inhibition of the microorganisms collected.

The pocket A between the layers of the window member 4 receives a filter element or membrane 6. A filter membrane is selected which is isoporous and which has a pore configuration suitable for the quantitative retention of microorganisms. However, the filter is permeable to air and liquids. An example of a satisfactory filter membrane is one which meets the requirements for the test of *E. Coli*. Such a microfilter may have, but is not limited to, a Z-value of 1–12 seconds; a thickness of .1–.15 mm., a diameter of 5 cm. with an active filter area of 9–9.5 $cm.^2$ and is thus capable of passing a test quantity of water of 500 cc. in about 1 min. with passing less than .01% of the Coli bacteria present in the sample.

The Z-number of a microfilter is defined as follows:

Z is the number of seconds required for 1 $cm.^3$ of H_2O at 22° C. to pass quantitatively through a $cm.^2$ of micro-filter area at a differential pressure of 70 cm. (700 mm.) Hg. Similar conditions hold for air filtration if the respective viscosities are considered. The critical condition is the retention of the microorganism on the surface of the filter.

The filter membrane is provided with a rim 7 which may be metal or plastic and serves to give rigidity to the membrane as well as protect the microorganism gathering side of the filter membrane from contact as will be brought out hereinafter.

If a metal ring is employed, it should be properly lacquered in order to protect the surface against chemical attack and subsequent growth-inhibitory action on the organism on the membrane.

The attachment of this rim to the membrane filter is facilitated as follows: The membrane, preferably prior to trimming, is laid flat on a glass plate. The prepared rim is moistened with a solvent of a boiling point substantially higher than room temperature (in order to avoid too fast a drying of the solvent layer deposited on the rim).

A thin felt pad is impregnated with the solvent; for instance, butyl acetate, methyl alcohol, amyl acetate, or the like; the rim is then brought in contact with this felt pad in a manner similar to a rubber stamp and a stamp pad. The lacquer layer on the rim absorbs a thin solvent layer which, upon contact with the membrane, dissolves the latter superficially and causes permanent adhesion of the membrane to the rim.

The pocket A of the pocket structure also receives a circular pad 8 of absorbent material which may be formed of heavy blotting paper. A blotting paper must be selected which will not inhibit or interfere with the growth microorganisms, due to its soluble constituents, such as fillers, sulphites or similar bleaching chemicals. Its water capacity should be about 1 to 1.5 ml.+ of water for a disk of 50 mm. diameter and its pH must be adjusted to the particular nutrient.

The nutrient pad is pre-treated with a nutrient solution which is then dehydrated. For different purposes, different nutrient solutions are used, and effectively the pad may be dyed an identifying color. The nutrient pad is, of course, dehydrated under conditions which are not destructive to the components of the nutrient.

The dehydrated and sterile absorbent pad as well as the sterilized filter membrane are placed in the pocket, and the end of the pocket is folded and sealed. In this condition, the filter membrane and nutrient pad may be stored and transported until ready for use; in fact the assaying units or assemblies may be conveniently carried on the person of the operator.

The entire assembly is preferably sterilized before folding or sealing the contents of the envelope. This may be accomplished with a gaseous disinfectant such as ethylene oxide.

To utilize the assaying assembly for the assaying of aerosols an adaptor fitting 9 is employed which comprises a conical member having a stem for attachment to a hose or vacuum pump not shown. The vacuum pump may be hand operated or mechanically operated. The receiving or larger end of the conical portion of the adaptor is provided with a counterbore which forms a shoulder on which is fitted a porous plate 10, having a metal rim 11. The upper surface of the plate and rim is disposed flush with the upper surface of the face of the fitting to receive the filter membrane 6 with rim 7 uppermost. A portion of the upper face of the adaptor 9 is provided with an arcuate retainer flange 13 which overlies and holds the rim 7. Diametrically opposite from the flange 13 is a spring clip 14 which coacts therewith to hold the filter membrane in place. The plate 10 is isoporous and is provided with a porosity substantially greater than the microfilter.

My method of microbiological assaying of aerosols involves placing the above-mentioned rimmed microfilter membrane in the fitting 9 and drawing air to be sampled inwardly through the fitting 9 so that the air passes through the filter and any microorganisms present in the air are collected on the outer surface of the membrane. For comparative purposes a predetermined quantity of air is passed through the filter.

The nutrient pad is then impregnated to its full capacity with water. The pad is then placed on the under side of the filter membrane; that is, on the side opposite from the rim 7. The moistened nutrient pad and filter membrane are then returned to the pocket structure and the end 5 folded, care being taken not to touch the

surface of the membrane. Alternatively and more conveniently, the pad 8 may remain in its pocket and the requisite water added by an eye dropper or the like, care being taken not to supply too much water.

The pocket structure holds the nutrient pad in such initimate physical wetting contact with the microfilter membrane that the nutrient solution may enter and permeate the filter membrane and supply microorganisms present on the outer surface thereof as represented diagrammatically in Figure 9. The pocket structure also serves to facilitate handling without contaminating microfilter, both prior and after filtration.

The rim 7 serves to space the window or transparent sheet 4 from the surface of the microfilter membrane so that an air space is provided for the microorganisms, and physical contact therewith is avoided. Also the rim minimizes the direct wetting of its upper or collecting surface by accidentally free water in the pocket. Still further the rim facilitates handling of the microfilter both before and after use while precluding danger of contamination by contact with the membrane. The window permits observation of any colony growth without unsealing the pocket structure.

While sterile water may be used to moisten the nutrient pad, and is preferred in some cases, this is not always necessary for the reason that the intimate contact between the microfilter membrane and the nutrient pad prevents growth of the type of microorganism requiring the presence of oxygen (aerobic organisms). Facultative anaerobes which also propagate in the absence of oxygen are not as common. Furthermore such anaerobes are easily distinguished and, more important, such bacteria can not pass upwardly through the microfilter membrane so that the specimen microorganisms on the upper side of the membrane remain isolated.

It should, of course, be noted that care is exercised to avoid contact with the collecting surface of the microfilter membrane during the processes of obtaining a sample. The assaying cell is sufficiently small and relatively well protected by the pocket structure so that a large number may be easily carried on the person of the operator, provided that care is taken that no pressure is exerted on the window of the cell to cause it to cave in and contact the surface of the microfilter.

This has the further advantage that the body heat of the operator himself may serve to effect incubation of the micro-organisms contained in the exposed cells, where elevated temperature is necessary.

The apparatus for adapting the collecting unit or assembly to the assaying of hydrosols is shown in Figure 8.

An adaptor fitting 21 is provided with an upper face counterbored to receive a porous supporting plate 22 similar to the plate 10. The lower side of the fitting is constricted to form a discharge tube 23. The fitting is provided with a marginal flange which journals a collar 24. The microfilter is adapted to be positioned on the upper surface of the adaptor and its rim 7 is engaged by a gasket 25 such as an O ring set in the lower end of a measuring funnel 26. The funnel 26 and collar 24 are provided with mating threads or other means to clamp the filter in place.

Collection of a sample for a hydrosol consists in placing a measured amount of the liquid to be tested in the funnel 26, then permitting flow by

gravity or by aid of suction applied to the outlet tube 23 of the fitting 21.

After collection of the sample, the procedure is essentially the same as that employed in the assaying of aerosols. That is, the pad is properly wetted, placed under the microfilter, and returned to the cell.

Having thus described certain embodiments and applications of my invention, I do not desire to be limited thereto, but intend to claim all novelty inherent in the appended claims.

I claim:

1. A microbiological assaying cell, involving: a foldable and sealable pocket member having a transparent window; a microfilter having a raised rim; a nutrient pad adapted to be held by said pocket member in intimate contact with the side of said filler opposite from said rim, said rim spacing the window of said pocket member from said microfilter; said window permitting observation of microorganism colonies on said microfilter.

2. A microbiological assaying cell, involving: a substantially isoporous filter membrane permeable to gases and liquids, but having a pore configuration for quantitative retention of microorganisms carried by fluids passed through said filter membrane; and an initially dry absorbent pad impregnated with a microorganism nutrient, placed in contact with the other surface of said filter membrane to supply nutrient to the microorganism retained on said one surface when said absorbent pad is wetted.

3. A microbiological assaying cell, involving: a filter membrane substantially impermeable to air borne microorganisms but permeable to air and to liquids, whereby on drawing a quantity of microorganisms containing fluid through said membrane, specimen microorganisms are collected on one surface of said filter membrane; and a microorganism nutrient absorbent pad in contact with the surface of said filter membrane opposite from that on which said specimen microorganisms are deposited to supply nutrient through said membrane when said absorbent pad is wetted, said membrane excluding such microorganisms as may be present in said absorbent pad to prevent contamination of said specimen microorganism.

4. A microbiological assaying cell, involving: a filter membrane permeable to fluids, but having a pore configuration for quantitative retention of microorganisms carried by fluids passed through said filter membrane; and an initially dry absorbent pad impregnated with a microorganism nutrient, in contact with the other surface of said filter membrane to supply nutrient to the microorganisms retained on said one surface when said absorbent pad is wetted; and a foldable and sealable pocket member constructed and arranged to receive said filter membrane and absorbent pad prior to as well as after use, said pocket member having a transparent window to facilitate observation of colony growth, if any, of said specimen microorganisms.

5. A microbiological assaying cell, involving: a filter membrane substantially impermeable to air borne microorganisms but permeable to air and to liquids, whereby on drawing a quantity of microorganisms containing air through said membrane, specimen microorganisms are collected on one surface of said filter membrane; and a microorganism nutrient treated absorbent pad in contact with the surface of said filter membrane opposite from that on which said specimen microorganisms are deposited to supply nutrient through said membrane when said absorbent pad is wetted, said membrane excluding such microorganisms as may be present in said absorbent pad to prevent contamination of said specimen microorganisms.

6. A microbiological assaying cell, involving: a filter membrane permeable to fluids, but having a pore configuration for quantitative retention of microorganisms and arranged so as to collect on one of its surfaces microorganisms carried by fluids passed through said filter membrane; and an initially dry absorbent pad impregnated with a microorganism nutrient, in contact with the other surface of said filter membrane to supply nutrient to the microorganisms retained on said one surface when said absorbent pad is wetted; a rim disposed on the specimen microorganism collecting side of said filter membrane; a foldable and sealable pocket member adapted to receive said filter membrane and absorbent pad prior to as well as after use, said pocket member adapted to maintain said absorbent pad and filter membrane in intimate contact; said pocket member having a window overlying the microorganism specimen side of said filter membrane and spaced therefrom by said rim permitting observation of colony growth, if any, of said microorganism specimens.

7. A microbiological assaying cell, involving: a filter membrane substantially impermeable to microorganisms but permeable to fluids, whereby on drawing a quantity of microorganism containing fluid through said membrane, specimen microorganisms are collected on one surface of said filter membrane; and a microorganism nutrient treated absorbent pad in contact with the surface of said filter membrane opposite from that on which said specimen microorganisms are retained to supply nutrient through said membrane when said absorbent pad is wetted, said membrane excluding such microorganisms as may be present in said absorbent pad to prevent contamination of said specimen microorganisms; a rim disposed on the specimen microorganisms collecting side of said filter membrane; a foldable and sealable pocket member adapted to receive said filter membrane and absorbent pad prior to as well as after use, said pocket member adapted to maintain said absorbent pad and filter membrane in intimate contact; said pocket member having a window overlying the microorganism specimen side of said filter membrane and spaced therefrom by said rim permitting observation of colony growth, if any, of said microorganism specimens.

ALEXANDER GOETZ.

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