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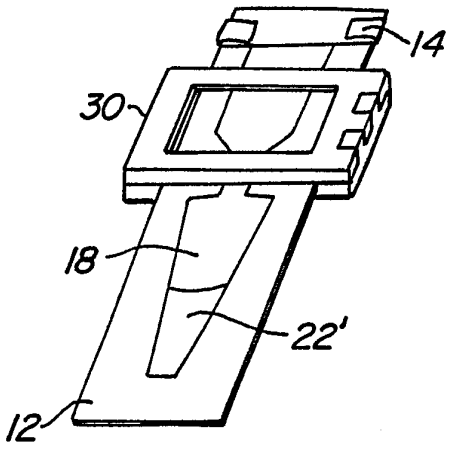

(57) Abstract

A culture system for microorganisms comprises a flexible pouch (12) having at least a first (6) and second (18) chamber for containing a culture medium (22). Biological specimen is first dispersed in a limited amount of the culture medium (22″) present in the first chamber. The newly-collected specimen may by immediately viewed under the microscope for the presence of the target microorganism. The inoculated portion of the culture medium (22″) may then be transferred to the second chamber (18) for culturing of the microorganism over time. Such subsequent culturing is particularly useful for allowing the target microorganism to proliferate to a visually detectable level.

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METHOD AND SYSTEM FOR DETECTING AND CULTURING MICROORGANISMS

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to the detection and culturing of microorganisms present in biological samples. More particularly, the present invention relates to the use of a flexible pouch structure which allows for collection and initial screening of a biological sample in a first chamber and subsequent culturing and screening of the sample in a second chamber.

The detection and culturing of microorganisms is of interest under a variety of circumstances. In particular, the collection and screening of patient specimens is essential to the proper treatment of a wide variety of infectious diseases suffered by humans and animals. Additionally, there is a need to be able to screen food, water, and other comestibles for contamination by pathogenic microorganisms, particularly bacteria.

One widely employed technique for microbiological screening relies on the initial culturing of a sample in a selective medium followed by visual, usually microscopic, examination to detect microorganisms which may have been present in the sample. Such techniques are advantageous in that they can combine the benefits of nutritional screening (which can suppress the growth of competing microorganisms) with the benefits of morphological confirmation of the presence of the target microorganisms. Such techniques, however, also suffer from certain limitations. The time required for culturing the sample can be as long as one day to several weeks. Such a delay is highly undesirable when treating an infection which requires immediate therapeutic intervention. Additionally, such screening techniques

often require that the sample be sent to a central reference laboratory, where the specimen is cultured, a colony of the cells is sampled and mounted on a microscope slide for examination. Each of these steps may involve the manual transfer of the specimen, requiring highly skilled personnel. Moreover, each transfer is an opportunity for contamination of the sample and/or release of pathogenic microorganisms into the environment.

For these reasons, it would be desirable to provide systems and methods for collecting biological specimens which allow for transporting, culturing, and direct observation of the specimen within a sealed environment. In particular, it would be desirable if the system allowed for collection of a specimen in the field and direct observation of the newly-collected specimens, preferably under a microscope. The system geometry should enhance the visual detectability of the microorganisms, particularly by concentrating the microorganisms within a portion of the system, and should further facilitate microscopic viewing of the microorganisms within the specimen container. It would be further desirable to provide a two-stage method wherein the newly-collected specimen could be observed. If no microorganisms were detected initially, the specimen could then be inoculated into a volume of culture medium sufficient to allow the microorganisms to proliferate to a detectable level.

2. Description of the Background Art

U.S. Patent No. 3,036,894 describes a multiple chamber testing container, where successive chambers are charged with reagents for reacting with a sample. Generally, the chambers are separated by frangible seals, but in a particular embodiment intended for serum titration, the device is a tubular body where successive chambers are connected by narrow tubes which may be either opened or closed. U.S. Patent No. 3,915,806,

discloses a flexible pouch suitable for transporting specimens, particularly specimens collected on swabs. The pouch includes adjacent chambers, where a first of the chamber holds a sterile swab. A second chamber includes a lower compartment which contains a transport medium in which the swab tip is immersed during transport. U.S. Patent No. 3,776,220 discloses a swab transport tube containing a medium which may be a culture medium. U.S. Patent No. 3,660,033 discloses a specimen collection and analysis bag including several chambers, two of which may be connected by an open channel.

Specimen collection bags sold under the trade names Whirl-Pak™ and Trans-Pak™ are commercially available from Nasco, Fort Atkinson, Wisconsin. The specimen collection bags include a single compartment which may be opened at the top and includes a wire closure for resealing.

A plastic envelope culture system manufactured by International Health Services, East Palo Alto, California (assignee of the present application), is described in Ching et al. (1988) Genitourin Med. 64:180-184. The IHS culture system includes a single rectangular screening chamber containing culture medium suitable for culturing and subsequent microscopic examination.

Other specimen collection and/or culture systems are described in the following U.S. patents: 4,690,801; 4,645,486; 4,330,627; 4,294,582; 4,241,045; 4,196,167; 4,013,422; 3,853,127; 3,842,166; 3,776,220; 3,713,779; 3,697,227; 3,662,928; 3,572,340; 3,512,524; 3,476,515 (Re. 29,725); 3,221,741; 3,184,395; 3,122,480; 3,105,613; 3,009,498; 2,690,179; and 2,653,744.

SUMMARY OF THE INVENTION

The present invention comprises novel systems and methods for detecting and culturing microorganisms present in biological specimens. Systems include a flexible enclosure or "pouch" having at least a first

chamber and a second chamber formed therein. A preselected volume of culture medium is contained in one or both of the chambers, and the two chambers are connected by an open channel which permits transfer of the culture medium (or other fluid) therebetween. The channel, however, is dimensioned to inhibit flow between the chambers in the absence of external pressure to cause flow from the pressurized chamber to the other chamber. In this way, the specimen and culture medium can be effectively contained within either of the chambers, while selective transfer between the chambers can be effected when desired. The use of a channel to separate the chambers is superior to a frangible seal in at least two respects. First, the channel is much easier to manufacture, avoiding problems associated with frangible seals that have widely varying strengths. Second, the channel allows containment to be maintained even after fluid has been transferred from one chamber to the other. Such continuing isolation is generally not available with frangible seals which, when broken, freely permit flow in either direction.

In the method of the present invention, a biological specimen is introduced to the first chamber. Usually, the flexible closure is opened (typically by tearing off one end), the sample is introduced, and the enclosure is resealed. A portion of the culture medium will normally be transferred to the first chamber prior to introduction of the specimen, although this will not always be necessary with liquid specimens. In the case of a swab sample or scraping, the specimen will be transferred by immersing the collection device into the culture medium within the first chamber to disperse the specimen therein.

Once the specimen has been introduced to the first chamber and the enclosure resealed, the newly-collected specimen may be examined substantially immediately, i.e., without allowing time for

proliferation. Usually, the first chamber will be configured to collect and concentrate the biological specimen in a relatively small portion thereof. Typically, the liquid volume within the first chamber will be limited in order to increase the concentration of the microorganism and enhance the detectability thereof.

If the initial examination fails to detect the target microorganism, the system of the present invention provides for subsequent culturing of the specimen to allow for proliferation to a detectable level. The specimen, typically suspended in the limited volume of culture medium, is transferred to the second chamber by squeezing or rolling down the first chamber to cause flow through the connecting channel. A sufficient volume of culture medium is present in the second chamber to allow for proliferation of microorganisms above a detectable threshold concentration. Usually, the second chamber will also be configured to concentrate the microorganism in a relatively small portion thereof. Typically, the chamber will be tapered so that such concentration will occur by suspending the enclosure vertically prior to examination, usually during the entire period of incubation. Optionally, the pouch may be periodically agitated to promote proliferation and settling of the organisms.

In the preferred embodiment, a viewing device is provided to facilitate examination of the contents of the enclosure under a microscope. The device comprises a pair of frames which are hinged together at one end. By clamping the frames about the portion of the enclosure which is to be viewed, the medium therein is entrapped. Thus, a volume of the culture medium having the concentrated microorganism present may be held in place as the medium is inspected under the microscope.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a perspective view illustrating a flexible enclosure constructed in accordance with the principles of the present invention.

Fig. 2 is a viewing device suitable for use with the flexible enclosure of Fig. 1.

Figs. 3-7 illustrate the use of the flexible enclosure of Fig. 1 and viewing device of Fig. 2 in the method of the present invention wherein a biological specimen collected with a swab is used to introduce the specimen to the enclosure.

Fig. 8 illustrates a flexible enclosure constructed in accordance with the principles of the present invention and including separate culturing chambers which contain different media.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The present invention is useful for detecting and culturing a wide variety of microorganisms which may be present in numerous types of biological specimens. The microorganisms will typically be human or animal pathogens which may be visually detected, usually under a microscope. The microorganisms will be capable of growth on a selective or non-selective culture medium, as described in more detail hereinbelow, and visual detection of the microorganisms may occur either before or after proliferation in the culture medium. Exemplary bacteria, yeast, fungi, and protozoa which may be detected using the culture system and method of the present invention are listed in Table 1 as follows.

TABLE 1

EXEMPLARY MICROORGANISMS

Bacteria	Associated Disease
Neisseria gonorrheae	sexually transmitted disease
Mycoplasma hominis	" "
Ureaplasma urealyticum	" "
Haemophilus ducreyi	" "

Gardnerella vaginalis	sexually transmitted disease
Campylobacter species	" "
Mobiluncus curtisii	" "
Neisseria species	meningitis
Bordetella pertussis	whooping cough
Brucella species	brucellosis
Salmonella typhi	typhoid fever
Salmonella typhimurium	gastroenteritis; septicemia
Salmonella schottmulleri	" "
Salmonella choleraesius	" "
Shigella dysenteriae	bacterial dysentery
Staphylococcus aureus	toxic shock, infections
Streptococcus pyogenes	streptococcal infections
Streptococcus agalactiae	sexually transmitted disease
Vibrio cholerae	cholera
Yersinia pestis	plague
Pseudomonas aeruginosa	wound and burn infection
Escherichia coli	urinary infection
Klebsiella pneumoniae	respiratory, urinary infection
Clostridium species	gangrene; tetanus
Yeast	
Candida albicans	yeast infection
Candida tropicalis	" "
Candida parasilosis	" "
Candida guilliermondii	" "
Cryptococcus neoformans	" "

Fungi	
Epidermophyton species	skin fungus
Trichophyton species	" "
Microsporum species	" "
Coccidiodies immitis	Valley Fever
Protozoa	
Trichomonas foetus	Bovine trichomoniasis
Trichomonas vaginalis	Sexually transmitted disease
Trypanosomes	Sleeping sickness

The present invention provides for the dispersion and culturing of the target microorganism in a culture medium capable of supporting growth of said microorganism. The culture medium will be a liquid as it will usually be necessary to flow the medium between chambers in the culture device, as described in more detail hereinbelow. Usually, the medium will consist primarily of water and will include at least one organic carbon source as well as the minerals necessary to support growth of the target microorganism. Normally, the medium will be selective for growth of the target microorganism, i.e., it will provide for preferential growth of the target microorganism over competing microorganisms which might be present in the biological specimen. In some cases, however, it may be possible to employ non-selective media, particularly when few or no competing microorganisms are suspected to be present. Particular selective media for the various bacteria, yeast, fungi, and protozoa listed in Table 1 are well described in the patent and scientific literature.

A culture medium which is particularly suited to support the growth of *Neisseria gonorrheae* is set forth in Table 2. This medium has been found to support rapid growth of *N. gonorrheae* under appropriate temperature conditions (35° - 37°C), allowing

proliferation of the cells to occur in the laboratory environment or during transportation of the culturing system to a central testing laboratory. Other organisms may require different temperature conditions.

TABLE 2

CULTURE MEDIUM FOR *N. GONORRHEA*

Component	Amount
polypeptone peptone BBL	15.0 g
cornstarch	1.0 g
yeast extract	5.0 g
glucose	5.0 g
Na_2HPO_4	5.0 g
NaH_2PO_4	2.0 g
KCl	5.0 g
$NaHCO_3$	10.0 g
Iron Dextran (Imferon)	20 mg
growth factors	As necessary
vancomycin	1.5 mgm
polymixin B	7.5 mgm
trimethoprim	8.0 mgm
sulfamethoxazole	2.0 mgm
distilled water to make	1.0 liter

Growth medium suitable for the culturing of pathogenic yeasts, including *Candida albicans*, is set forth in Table 3. This culture medium will allow the proliferation of *C. albicans* at ambient temperatures in the culture system of the present invention.

TABLE 3

CULTURE MEDIUM FOR YEAST

Component	Amount
Soytone	9.6 g
Dextrose	9.6 g
NaH_2PO_4	2.0 g
Na_2HPO_4	1.0 g
Chloramphenicol	0.05 g
Water to make	1.0 liter

A growth medium suitable for at least some protozoa, *Trichomonas foetus* and *Trichomonas vaginalis*, is set forth in Table 4. The temperature conditions for supporting growth will vary with the nature of the protozoa.

TABLE 4

GROWTH MEDIUM FOR PROTOZOA

Component	Amount
Trypticase	9.0 g
Proteose peptone	6.0 g
Liver extract	3.0 g
Yeast extract	10.0 g
Maltose	4.0 g
KCl	0.90 g
NaH_2PO_4	1.90 g
Na_2HPO_4	0.90 g
L-cystein	1.00 g
L-glutamic acid	0.10 g
$KHCO_3$	0.90 g
Ascorbic acid	0.60 g
Vitamin B-12 (1000 mg/ml)	0.20 ml
Chloramphenicol	0.16 g
Horse serum	100 ml
Water to make	1.0 liter

The present invention is suitable for screening a wide variety of biological specimens, including virtually any substance capable of supporting a target microorganism in a viable condition. Biological specimens of particular interest include human patient samples and veterinary samples, such as blood, serum, plasma, urine, cerebral fluid, spinal fluid, ocular lens liquid (tears), saliva, pharyngeal exudate, sputum, semen, urethral discharge, cervical mucus, scrapings, swab samples, and the like. Other specimens of interest include water, soil, food samples, particularly including

meat and dairy products which are susceptible to contamination with pathogenic microorganisms. Patient and veterinary specimens may be obtained by swabbing, scraping, drawing blood, biopsy, lavage, and other common medical specimen collection techniques. Water and soil samples are commonly collected by well known environmental sampling techniques.

In addition to growth media and other liquid dispersants, the flexible enclosure of the present invention may contain a wide variety of reagents capable of interacting with the target microorganisms in some desired manner, usually by providing a detectable signal or other indication that the target microorganism is present. Such reagents include immunological substances, e.g., antibodies, antigens, haptens, and the like; labels, e.g., enzymes, fluorescers, enzyme substrates, radiolabels, spin labels, and the like; bacteriophage specific for the target microorganism; etc. The reagents may be present in each of the chambers in the flexible enclosure or may be isolated within particular chambers, depending on the desired detection protocol.

Referring now to Fig. 1, a collection and culturing device 10 constructed in accordance with the principles of the present invention includes a flexible enclosure or pouch 12 having a seal strip 14 at one end thereof. The enclosure 12 is formed by a pair of optically transparent sheets which are heat sealed together in a particular pattern selected to define a first chamber 16 and a second chamber 18 therein. The first chamber 16 and second chamber 18 are connected by a channel 20 which allows transfer of a liquid culture medium 22 between the two chambers. Two pull tabs 19 (only one of which is visible in Fig. 1) are secured to opposite sides of the seal strip 14 to facilitate opening of the first chamber 16, as described in more detail hereinafter.

The individual sheets which form the enclosure 12 will be formed from a liquid-impermeable material which is capable of retaining the culture medium with little or no evaporation to the ambient. For anaerobic microorganisms, the material will also be oxygen-impermeable in order to maintain an oxygen-depleted environment in the chambers 16 and 18. For aerobic microorganisms, it may be desirable to form at least a portion of the enclosure 12 from an oxygen permeable material, such as silicone rubber, or the like, in order to allow oxygen penetration to support growth of the microorganism. Alternatively, oxygen for aerobic microorganisms may be provided by leaving the top of the enclosure 12 open or by leaving substantial air in the upper chamber 16.

Generally, the individual sheets which form the enclosure 12 will be multiple layer structures including a tough outer layer for resisting penetration and tearing, an oxygen barrier layer (if desired) and an inner heat sealing layer. Particularly suitable for the outer protective layer are polyethyleneterephthalate (PET), biaxially oriented nylon (BON), and the like. Suitable oxygen barrier layers include polyvinyl dichloride (PVDC), ethylene-vinyl alcohol (EVOH), and the like. Suitable heat seal layers include linear low density polyethylene (LLDPE), ionomer (derivatized ethylene-vinyl copolymer), and the like.

The flexible enclosure 12 may thus be formed by heat sealing the layers together about the periphery, leaving the internal chambers 16 and 18 and connecting passage 20 unsealed. The external periphery of the enclosure 12 may be trimmed either before or after heat sealing of the layers together.

The dimensions of the enclosure 12 are not critical. Usually, enclosure 12 will have a length in the range from about 10 to 30 cm and a width in the range from about 2 to 10 cm. The first chamber 16 will

normally be rectangular with a tapered end connecting to the channel 20. The width of the chamber 16 will usually be in the range from about 1 to 8 cm (prior to tapering) and the length in the range from about 5 to 15 cm. The interior of chamber 16 is expandable to hold a liquid culture medium and will usually have a capacity in the range from about 0.5 to 10 ml, although a chamber will usually not be filled to capacity.

Second chamber 18 will usually have a tapered configuration, with the width of the chamber decreasing in the direction away from the connection to channel 20. The width of chamber 18 will generally be from about 1 to 8 cm at its top (i.e., the end proximate the channel 20) while being from 0 to 5 cm at the bottom. The length of chamber 18 will generally be from about 5 to 15 cm, with the taper being formed by a pair of converging straight edges. As with the first chamber 16, the second chamber 18 will expand to contain the liquid culture medium 22, having a capacity generally in the range from about 0.5 to 10 ml.

The dimensions of the connecting channel 20 are selected to inhibit the flow of culture medium 22 therethrough. Generally, channel 20 will have a length which exceeds its width, with the length usually being within about 0.5 to 5 cm and the width being in the range from about 0.2 to 2 cm.

As illustrated in Fig. 1, the chambers 16 and 18 and the channel 20 are completely sealed, preventing loss of the culture medium 22 from the interior. Usually, the culture medium will initially be present only in the second chamber 18, although any desired portion of the medium may be transferred to the first chamber 16 by passage through the connecting channel 20. Such transfer is effected by simply squeezing the exterior of enclosure 12 in the region proximate the second chamber 18. In this way, the culture medium 22 is forced to flow through the channel 20. The culture

medium may also be transferred in the opposite direction by squeezing or rolling down the first chamber 16.

A tear strip 24 is provided at the top of flexible enclosure 12 to allow opening of the first chamber 16. Typically, the tear strip 24 is defined by a pair of notches 26 formed on opposite sides of the enclosure and may further include a thinned line or region which facilitates tearing across from notch to notch.

Referring now to Fig. 2, a viewing device 30 intended to mount a portion of the enclosure 12 for viewing under a microscope (not illustrated) is illustrated. The viewing device includes a pair of frame members 32 which are hinged together at one end so that the device 30 may be clamped across the enclosure 12, as described in more detail hereinafter. A pair of posts 34 at the non-hinged end of one of the frames 32 mate with a pair of receptacles 36 formed in the other frame. The openings 38 formed in each of the frames 32 are sized to extend across the enclosure 12 to allow unimpeded examination of the contents of the enclosure when it is held in the device 30.

Referring now to Figs. 3-7, use of the culture system 10 in the method of the present invention will be described. Usually, a portion of the culture medium 22' is transferred from the second chamber 18 to the first chamber 16. The volume of culture medium 22'' (Fig. 3) transferred is selected to be sufficient to disperse the biological specimen while being sufficiently small to not overly dilute any microorganisms which may be present. Generally, the transferred medium 22'' will have a volume in the range from about 0.2 to 5 ml, more usually being in the range from about 0.5 to 2 ml.

After the culture medium 22'' is transferred to first chamber 16, the tear strip 24 is removed from enclosure 12 and the upper portion of first chamber 16 opened by pulling apart its two sides using tabs 19, as

illustrated in Fig. 4. The biological specimen is then introduced to the culture medium 22''. As illustrated, a collection swab is immersed in the culture medium 22''. Other methods for introducing both cellular and liquid specimens would also be suitable.

The volume of culture medium 22'' is generally maintained in the first chamber by the flow-restrictive nature of connecting channel 20. That is, the opposed faces of the flow channel 20 remain generally together, inhibiting flow back into the second chamber 18.

In the case of liquid or moist specimens, it may not be necessary to introduce any liquid into the chamber or the amount of liquid required may be substantially diminished. Alternatively, for all specimens, it may be possible to introduce a liquid dispersant (other than culture medium) into the first chamber 16. Such dispersant may be introduced at the time the enclosure is fabricated or may be introduced by the user after opening.

After the biological specimen is introduced to the culture medium 22'' in the first chamber 16 (or introduced without culture medium), the upper end of enclosure 12 will be sealed using the sealing strip 14 to fold over the open end and hold it in place, as illustrated in Fig. 5. Usually, the enclosure 12 will be suspended vertically for a period of time sufficient to allow microorganisms to collect in the tapered lower region of chamber 16. A period in the range from 5 minutes to 1 hour will be normally sufficient, more usually in the range from about 10 to 20 minutes. Once the microorganisms have had a chance to collect in the first chamber 16, the viewing device 30 will be clamped about the chamber 16, as illustrated in Fig. 5, and the liquid medium uniformly dispersed within the region defined by the window of said viewing device. The viewing device 30 can then be placed horizontally on a microscope stage for viewing in a conventional manner.

The technician examining the contents of the first chamber 16 will look for microorganisms having the known characteristics of the target microorganism. If the inoculum has a sufficient amount of microorganisms, e.g., above 10^7/ml, viewing may be accomplished without prior vertical suspension of the enclosure 12.

Detection of the target microorganism in the first chamber 16 may be all that is desired in certain screening applications. In other screening applications, however, it will be desirable or necessary to further culture the microorganism in the second chamber 18. Moreover, when examination of the first chamber 16 fails to reveal the presence of the target microorganism, it will usually be desirable to inoculate the culture medium 22' in the second chamber 18 with the contents of the first chamber in order to allow the target microorganisms, if present, to proliferate to a detectable level.

Such further culturing of the target microorganism is accomplished by transferring at least a portion of the contents of the first chamber 16 back into the second chamber 18. As described above, this is effected by squeezing or rolling down the first chamber 16 to force the culture medium and any microorganisms present through connecting channel 20 into second chamber 18. Once in second chamber 18, the inoculated culture medium will recombine with the remaining culture medium 22' to form a greater volume of inoculated culture medium 22''' (Fig. 6). The inoculated culture medium 22''' will then be allowed to incubate until evidence of microbial growth is detected or for a preselected period of time, usually from about 2 hours to one month, more usually in the range from about 18 hours to one week. Such incubation is preferably carried out with the enclosure 12 suspended vertically so that the microorganisms will tend to collect at the bottom of the tapered second chamber 18. For some organisms, such as *N. gonorrheae*,

it is advantageous to periodically agitate the pouch in order to promote growth and aid in settling of the organisms. The flexible pouch design permits a variety of agitation techniques, such as striking the exterior of the pouch with a rod or immersing the pouch in an intermittently-agitated water bath. The latter may also be used for temperature control when desired.

For anaerobic or microaerophilic organisms, it will usually be desirable to force out as much air as possible from the first chamber 16 and to then roll down the chamber until reaching the channel 20. This minimizes the opportunity for oxygen penetration into the second chamber 18. Moreover, air remaining in the first chamber 16 will generally be trapped within the rolls, further assuring substantially anaerobic or microaerobic conditions in the second chamber 18.

For aerobic microorganisms, it may be desirable to leave the top of the enclosure 12 open or to seal a substantial volume of air into the first chamber 16.

The present invention is particularly suitable for culturing anaerobic and microaerobic organisms. Separation of the first and second chambers 16 and 18 allows for excluding all or most oxygen from the second chamber 18. Moreover, the flexible nature of the enclosure 12 allows the user to expel oxygen from chamber 16 by manually or mechanically squeezing the side walls of the chamber to force out any air which may have entered. Any residual air remaining after squeezing may be entrapped by rolling the first chamber 16 to inhibit migration and penetration in the second chamber. The small amounts of air which might enter the second chamber 18 are generally not a problem as aerobic organisms present in the inoculant will usually consume the remaining air in a very short period of time. Growth of such aerobic organisms will then cease, allowing the target anaerobic organisms to proliferate to a detectable level.

After the preselected time, or after evidence of microbial growth has been detected, the second chamber 18 may be observed under a microscope using the viewing frame 30 in a manner analogous to that described above for viewing of the first chamber 16. The viewing frame is clamped about the narrowest portion of chamber 18 where the presence of the microorganism is suspected. The device 30 is then placed on a microscope stage and the contents of chamber 18 observed.

According to the present invention, two-stage screening of biological specimens has been achieved using a single culturing and detection device. The specimen need be inoculated in the culturing device only once and thereafter remains isolated within the system. Manipulation of culture medium carrying the target microorganisms is effected by squeezing the medium back and forth between the two viewing chambers which are utilized for the respective stages of detection. The total amount of culture medium in the enclosure is sufficient to allow for proliferation of the target microorganism to a detectable level, typically being in the range from about 0.5 to 10 ml, more typically being in the range from about 3 to 6 ml.

An alternate embodiment 50 of the culture system of the present invention is illustrated in Fig. 8. The system 50 includes a flexible enclosure 52 which is similar in most respects to system 10, differing primarily in that a first chamber 54 is connected to second and third chambers 56 and 58 by channels 60 and 62, respectively.

The advantage of having two (or more) "lower" chambers connected to a single "receiving" chamber is that a single specimen can be initially screened in the first chamber 54 and subsequently cultured in different culture mediums, usually being specific for the growth of different microorganisms, e.g., *Gardnerella* and *Trichomonas*. When selective media are employed in the

separate lower chambers 56 and 58, it will usually be desirable to provide a dispersion medium in the first chamber 54 which will not support the growth of the target microorganisms. In this way, the selective media will not be contaminated when the inocula are transferred from the first chamber.

Although the foregoing invention has been described in detail for purposes of clarity of understanding, it will be obvious that certain modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A device for the detection and culturing of microorganisms, said device comprising:

a flexible enclosure having at least a first chamber for receiving a specimen suspected of containing the microorganisms and a second chamber containing a medium suitable for culturing the microorganisms, wherein said first and second chambers are connected by an open channel which inhibits flow between the chambers but allows the medium to be transferred between the first chamber and the second chamber by the selective application of external pressure on the enclosure, wherein at least a portion of the first and second chambers is optically transparent to allow for microscopic examination of the contents of each chamber.

2. A device as in claim 1, wherein the flexible enclosure includes at least one additional chamber connected to the first chamber by an open channel.

3. A device as in claim 1, wherein the enclosure is a pouch including a pair of optically transparent plastic sheets which are laminated together in a pattern selected so that non-laminated regions define the first chamber, second chamber, and open channel.

4. A device as in claim 3, further comprising a tear strip at one end to permit opening of the first chamber.

5. A device as in claim 4, further comprising a foldable tap proximate the tear strip to facilitate resealing of the flexible enclosure.

6. A device as in claim 1, wherein the enclosure contains a volume of culture medium in the range from about 0.5 to 10 ml.

7. A device as in claim 6, wherein the culture medium is contained substantially entirely within the second chamber and wherein said enclosure is folded across the open channel to substantially prevent flow therethrough.

8. A device as in claim 3, wherein the first chamber and second chamber each have a maximum width in the range from about 1 to 8 cm, while the channel has a width in the range from about 0.2 to 2 cm and a length in the range from about 0.5 to 5 cm.

9. A device as in claim 8, wherein the width of the first chamber is tapered downward in the direction of the open channel, while the width of the second chamber is tapered downward in the direction away from the open channel.

10. A device as in claim 1, wherein at least one of the chambers contains a reagent capable of interacting with the microorganism.

11. A method for the detection and incubation of microorganisms, said method comprising:

(a) introducing a biological specimen into a first chamber of an enclosure, said first chamber containing a volume of fluid selected to permit visual detection of said microorganism;

(b) visually inspecting the contents of the first chamber to determine if said microoragnisms are present; and

(c) transferring at least a portion of the contents of the first chamber to a second chamber of the

enclosure, said second chamber containing a volume of fluid selected to allow proliferation of the microorganism.

12. A method as in claim 11, wherein the biological specimen is introduced by opening the first chamber, exposing the specimen to the fluid within the chamber, and sealing the chamber.

13. A method as in claim 11, wherein the volume of fluid within the first chamber consists of from about 0.2 to 5 ml of a medium capable of supporting growth of the microorganisms.

14. A method as in claim 11, wherein the contents are visually inspected with a microscope.

15. A method as in claim 11, further comprising visually inspecting the contents of the second chamber after a time sufficient for the microorganisms to grow to a detectable level.

16. A method for the detection and incubation of microorganisms, said method employing a flexible enclosure having a first chamber, a second chamber, a channel between said chambers, and a preselcted volume of growth medium transferrable between said chambers through the channel, said method comprising:

(a) apportioning the growth medium between said chambers so that the first chamber contains a volume of growth medium sufficient to form a visually detectable dispersion of microorganisms;

(b) introducing a biological specimen into the first chamber, whereby any microorganisms present are dispersed in the growth medium therein;

(c) visually inspecting the contents of the first chamber to determine if said microorganisms are present;

(d) if no microorganisms are detected in step (c), transferring at least a portion of the growth medium in the first chamber to the second chamber, wherein the combined volume of growth medium is sufficient to incubate the sample for a time sufficient to allow growth of the microorganisms to reach a visually detectable stage; and

(e) visually inspecting the contents of the second chamber after said sufficient time to determine if said microorganisms are present.

17. A method as in claim 16, wherein the growth medium is apportioned by squeezing or rolling down one of said chambers to force the growth medium through the channel to the other chamber.

18. A method as in claim 17, wherein a total volume of growth medium in the range from about 0.5 to 10 ml is contained in the enclosure and a volume in the range from about 0.5 to 5 ml is initially apportioned in the first chamber.

19. A method as in claim 16, wherein the biological specimen is introduced by opening the first chamber, exposing the specimen to the growth medium within the chamber, and sealing the chamber.

20. A method as in claim 16, wherein the biological specimen is selected from the group consisting of swab samples, scrapings, blood, serum, plasma, urine, cerebral fluid, spinal fluid, ocular lens liquid, saliva, sputum, semen, and cervical mucus.

21. A method as in claim 16, wherein the first chamber is rolled down to limit oxygen transfer to the second chamber after said portion of the growth medium is transferred.

22. A method as in claim 16, wherein the flexible enclosure is suspended vertically prior to visual inspection of the first chamber in order to allow said microorganisms to collect at one end thereof.

23. A method as in claim 16, wherein the flexible enclosure is suspended vertically prior to visual inspection of the second chamber in order to allow said microorganisms to collect at one end thereof.

24. A method as in claim 23, wherein the flexible enclosure is periodically agitated while suspended vertically.

25. A method as in claim 24, wherein the flexible enclosure is agitated by periodic striking or by immersion in an agitated bath.

26. A kit containing:

a flexible enclosure having a first chamber, a second chamber, a channel between said chambers, and a preselected volume of growth medium transferable between said chambers through the channel;

a viewing plate; and

instructions setting forth the following method using said flexible enclosure and viewing plate:

(a) introducing a biological specimen into the first chamber with a predetermined portion of the growth medium therein;

(b) affixing the viewing plate to the first chamber;

(c) microscopically examining the contents of the first chamber to determine if microorganisms are present;

(d) if no microorganisms are observed in step (c), transferring at least a portion of the growth medium in the first chamber to the remaining growth medium in the second chamber; and

(e) affixing the viewing plate to the second chamber after a time sufficient for the microorganisms to proliferate to a detectable level; and

(f) microscopically examining the contents of the second chamber to determine if microorganisms are present.

27. A kit as in claim 26, wherein the instructions further set forth that the growth medium is transferred to the second chamber by squeezing or rolling down the first chamber.

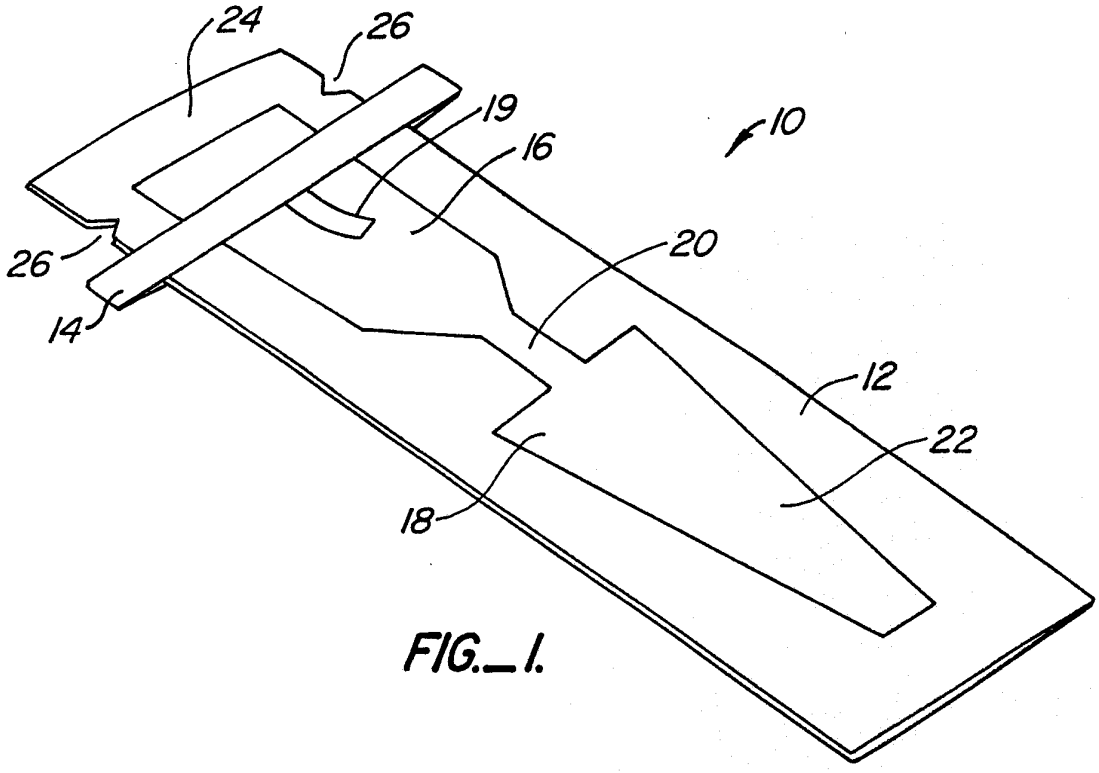

FIG._1.

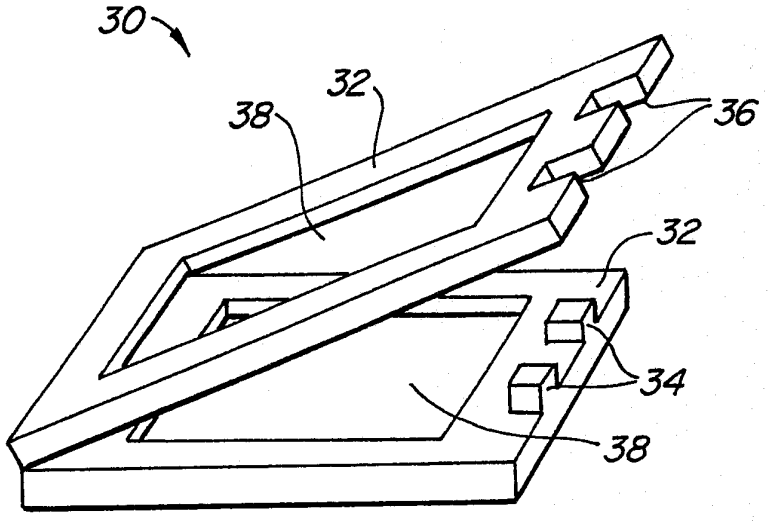

FIG._2.

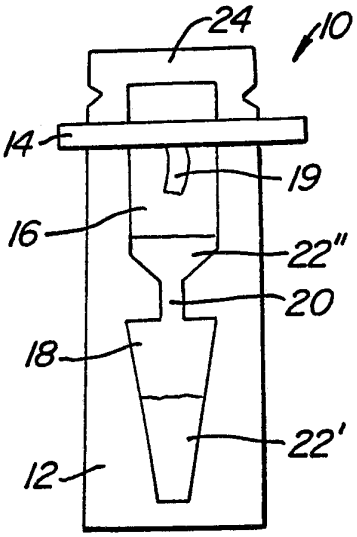

FIG._3.

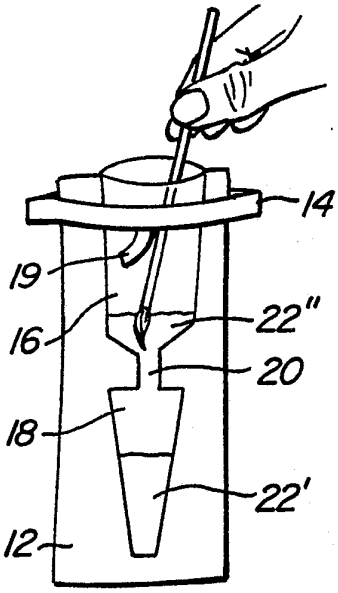

FIG._4.

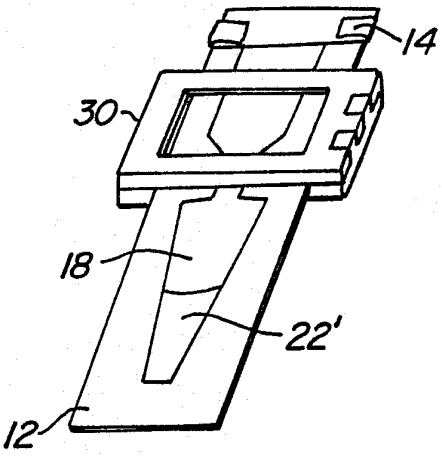

FIG._5.

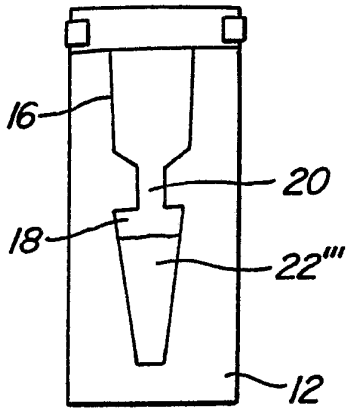

FIG._6.

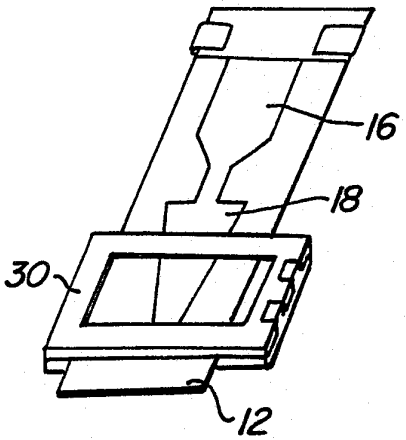

FIG._7.

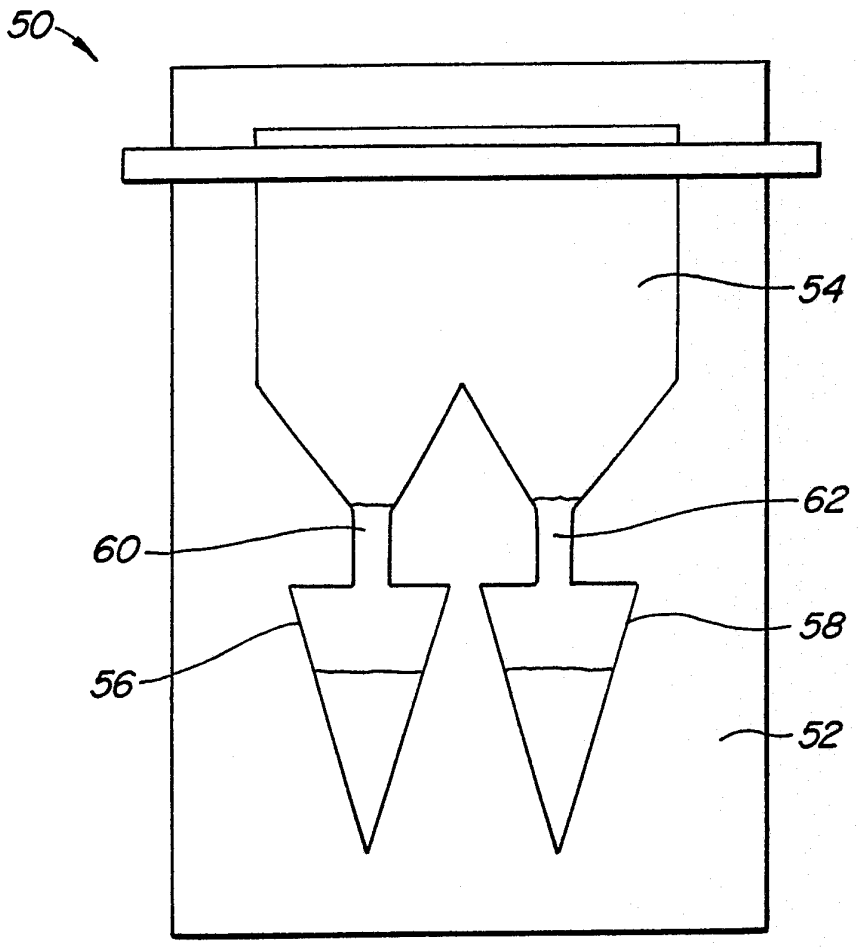

FIG._8.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/06605

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

Accrding to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C12Q 1/00

U.S. Cl: 435/29, 34, 293, 286, 285, 298, 301; 422/61

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	435/301, 298, 29-34, 293, 286, 285 422/61

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X ~~Y~~	US, A, 3,036,894 (FORESTIERE) 29 May 1962, see figures 4,5; column 1 lines 15-23; claim 1.	1,3-4 2,5-8,10-28
Y	US, A, 3,915,806 (HORLACH) 28 October 1975, see figures, col. 2 lines 8-13, col. 3 lines 13-27.	3-5,10,12,20, 23-25
X Y	US, A, 3,660,033 (SCHWARTZ) 02 May 1972, see figures, col. 4 line 30-col. 5 line 21, col. 6 lines 27-32.	1-3,8,9,11,12 7,10,13-25
A	US, A, 3,308,039 (NELSON) 07 March 1967.	1-25
A	US, A, 3,925,163, (CEKORIC, Jr. ET AL.) 09 December 1975.	1-25

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
13 December 1990	05 FEB 1991
International Searching Authority [1]	**Signature of Authorized Officer** [20]
ISA/US	William Chan

Form PCT/ISA/210 (second sheet) (May 1986)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . ... . , because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ..... , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

I. Device comprising two chamber pouch classified in class 435 subclass 293 (claims 1-10) and a method used to detect microorganism classified in class 435 subclass 34 (claims 11-25)

II. A kit comprising a pouch and view plate classified in class 422 subclass 61 (claims 26, 27).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

1-25 (Telephone Practice)

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.