United States Patent [19]

Bergeron

[11] **3,712,746**

[45] **Jan. 23, 1973**

[54] **DEVICE FOR OPTICALLY DETERMINING MICROBIAL COLONY POPULATION ON GROWTH SURFACE**

[75] Inventor: **John A. Bergeron,** Schenectady, N.Y.

[73] Assignee: **General Electric Company**

[22] Filed: **July 29, 1971**

[21] Appl. No.: **167,365**

[52] **U.S. Cl.** **356/256,** 356/36, 356/201, 356/208, 356/244, 356/246

[51] **Int. Cl.** **C12b 1/00,** C12k 1/00

[58] **Field of Search**.....356/36, 38, 102, 201, 208, 356/244, 246, 256

[56] **References Cited**

UNITED STATES PATENTS

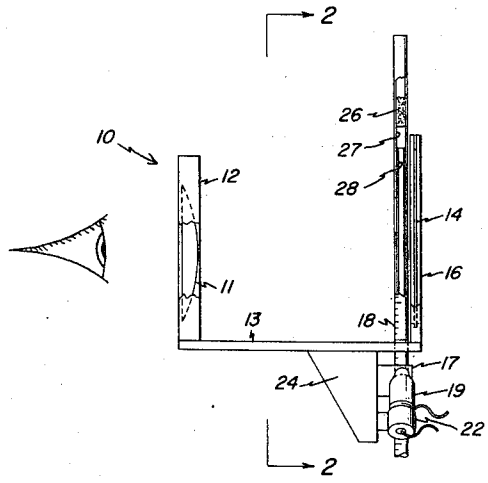
2,427,790	9/1947	Korman	356/208 X
2,157,438	5/1939	Sparks	356/208
2,495,912	1/1950	Wood	356/244

Primary Examiner—Ronald L. Wibert
Assistant Examiner—F. L. Evans
Attorney—Jerome C. Squillaro et al.

[57] **ABSTRACT**

A viewer-comparator device is described for optically determining the density of microbial colony growth in and on an inoculated nutrient layer. In the preferred construction the device comprises a base, a magnifying lens supported thereon, means connected to the base for supporting a nutrient layer-coated surface, this surface being located in position for viewing thereof through the lens, means to enable direct visual comparison by an observer between the appearance of the nutrient layer-coated surface and reproductions of microbial growth at a plurality of representative intervals over a range of growth densities of clinical interest and lighting means depending from the base. The comparison enabling means is located adjacent the position to be occupied by the nutrient layer-coated surface for simultaneous viewing of both the comparison enabling means and the coated surface through the lens. The lighting means are so directed that light rays emanating therefrom cannot pass to the lens by reflection or by direct passage from lighting means to lens. With the lighting means so disposed and directed, the microbial colonies will scatter the light reaching them and appear bright in an essentially dark field (i.e. no bright background).

6 Claims, 3 Drawing Figures



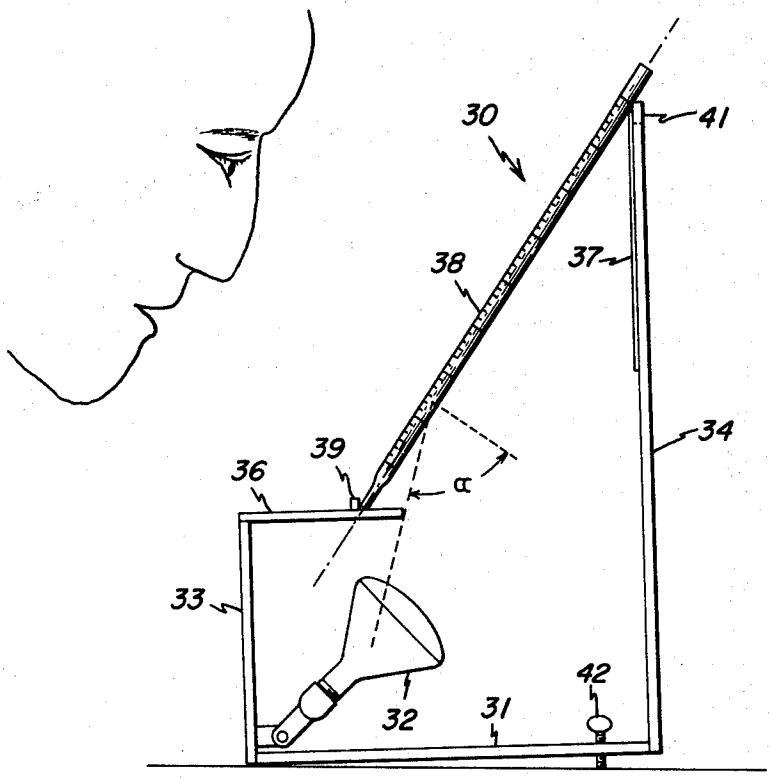


FIG. 3

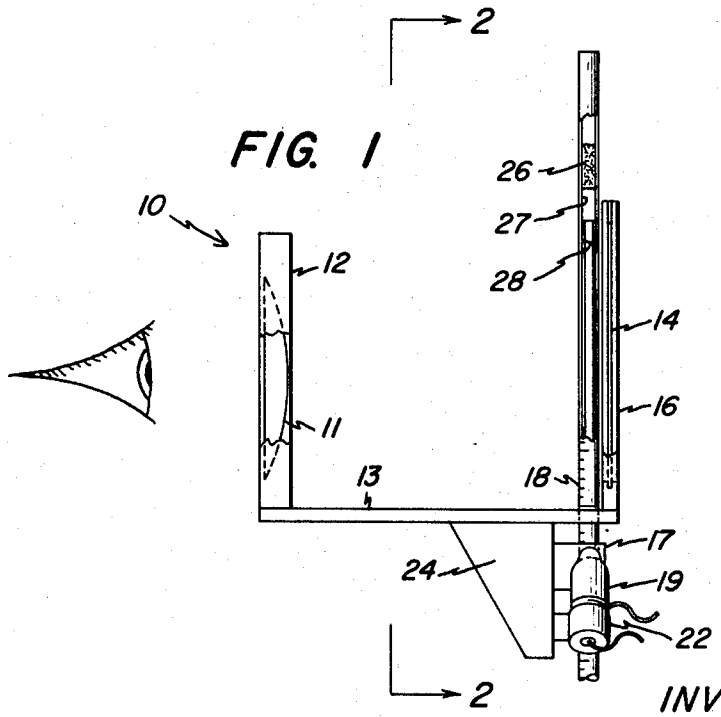
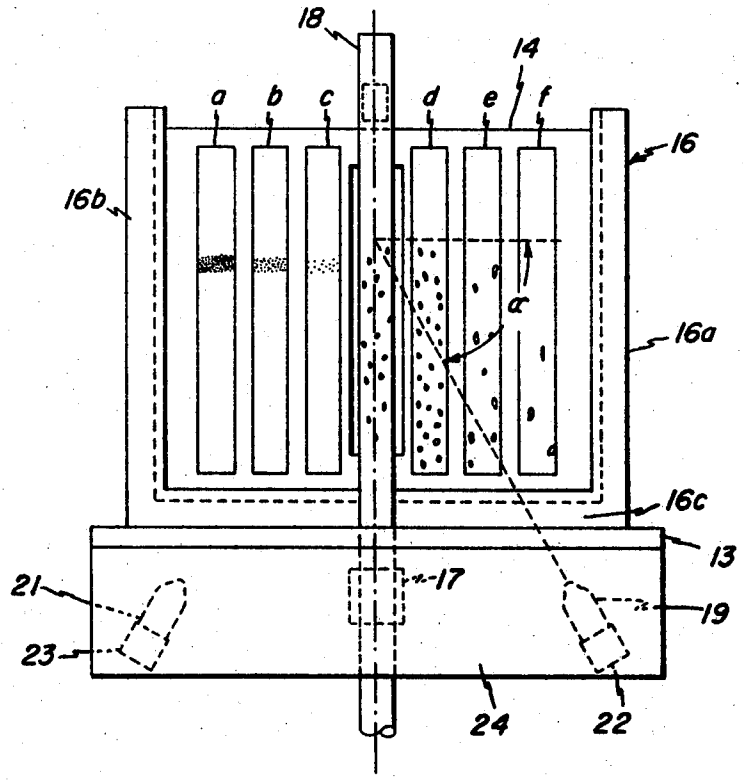


FIG. 1

INVENTOR:
JOHN A. BERGERON
by Leo S. Maloni
HIS ATTORNEY

FIG. 2



INVENTOR:
JOHN A. BERGERON
by Leon L. Mancuso
HIS ATTORNEY

DEVICE FOR OPTICALLY DETERMINING MICROBIAL COLONY POPULATION ON GROWTH SURFACE

BACKGROUND OF THE INVENTION

Improved devices are described in U. S. Patent application Ser. No. 130,254 — Bergeron for combined sampling and culturing to enable a measure of the microorganism content of a liquid sample. The aforementioned patent application is assigned to the assignee of the instant invention and was filed Apr. 1, 1971. The referred structure is a long, thin tube (e.g. a disposable sterile, transparent, pre-plugged serological pipette) having internal area thereof coated with a culture medium.

When such internally coated devices are used to determine the content of colony-producing microorganisms (i.e. bacteria, yeast or molds) in a liquid sample, e.g. urine, it is important to be able to quickly, easily and accurately determine the colony population density resulting from inoculation with the sample.

SUMMARY OF THE INVENTION

The above objective is satisfied by the viewer/comparator devices described herein for optically determining microbial colony population density on a transparent surface having an inoculated nutrient layer disposed thereon. In its simplest construction the device consists of a base, at least one light source, means for connecting this light source to the base, means connected to the base for positioning the transparent surface (e.g. the pipette of the aforementioned Bergeron application) and means for enabling direct comparison by an observer between the appearance of the transparent surface and reproductions of microbial growth at a plurality of representative intervals over some given range of clinical interest. The comparison-enabling means is located to permit simultaneous viewing of the transparent surface and the comparison-enabling means.

BRIEF DESCRIPTION OF THE DRAWING

The exact nature of this invention as well as objects and advantages thereof will be readily apparent from consideration of the following specification relating to the annexed drawing in which:

FIG. 1 is a side elevational view partially in section showing a magnifier-viewer-comparator constructed according to this invention and adapted to receive and hold a long, thin tube (e.g. a transparent, pre-plugged serological pipette) for visual examination;

FIG. 2 is a section taken on line 2—2 of FIG. 1 and

FIG. 3 is a side elevational view of a viewer/comparator device constructed according to this invention.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The magnifier-viewer-comparator device **10** is exemplary of a hand-held type unit constructed according to this invention and comprises magnifying lens **11** mounted in frame **12** supported upon base **13**, comparator card **14** mounted in open U-shaped frame **16** affixed to base **13**, U-shaped spring clamp **17** for releasably holding pipette **18** and a pair of light bulbs (e.g. GE–243 bulbs) **19, 21** mounted in sockets **22, 23**, respectively. The upright legs **16***a*, **16***b* and lower leg **16***c* of frame **16** are shown slotted to accommodate card **14**. Spring clamp **17** and sockets **22, 23** are affixed to mounting block **24** depending from base **13**.

Pipette **18** (more completely described in the aforementioned Bergeron application) is made of a transparent material, preferably a plastic. Plug **26** of cotton or similar fibrous material is disposed in passage **27** at the mouthpiece end of pipette **18**. The inner surface of passage **27** is covered by a layer **28** of a suitable solid (stiff gel capable of supporting itself) culture medium. Layer **28** will have been inoculated by contact with a liquid sample being checked for microorganism content by drawing some of the sample up into the nutrient-lined bore to a predetermined height below the upper limit of layer **28**. The liquid is then permitted to drain therefrom and after the tip of the pipette has been plugged, the unit is ready for colony development in the vertical position at room temperature or for incubation in a thermostatically controlled oven (not shown). Incubation is usually carried out at a temperature of about 37°C for a period of about 18–24 hours. After incubation (or standing at room temperature for the requisite period), the pipette **18** is ready for examination for microbial growth (e.g. colonies of bacteria) in device **10**. Pipette **18** is forced into the U-shaped spring clamp **17** and then slid endwise therein to a position adjacent comparator card **14** as shown in FIGS. **1** and **2**. If desired, the lower leg **16***c* of frame **16** may be slotted whereby with card **14** removed, pipette **18** may be directly positioned in clamp **17**.

In this position the pipette **18** is ready for magnified viewing along approximately a 2-inch length adjacent the junction between the inoculated and control portions of layer **28**. The control portion of this surface is, of course, that area of layer **28** remaining above the level to which the liquid sample had been raised in the pipette during inoculation. The growth characteristics in this 2-inch length provide the most information for the quantitative determination of microorganism content. Thus, it is of interest not only to be able to determine size and number of colonies, but also to be able to view the growth characteristics of the aforementioned junction.

The appearance of the pipette **18** may be readily compared to the standard displays: *a*, *b*, *c*, *d*, *e*, *f*, which are reproductions of growth characteristics of the microorganisms of interest at decadic intervals over the range of clinical interest (e.g. 10^3 – 10^8 cells/cc). Preferably comparator card **14** is slotted as shown behind the position to be taken by pipette **18**. The observer looking through lens **11** sees pipette **18** and the standards at some magnification (e.g. 2X), pipette **18** being located at or close to the focal length of lens **11**. In this manner, visual readouts for microorganism content may be readily obtained.

By way of illustration, urine specimens containing more than 10^5 viable bacteria/cc are generally considered to be from patients with an active infection of the urinary tract. At the lower concentration (up to and including 10^3 bacteria/cc) the pipette will appear substantially free of colonies. Similarly, at very high concentrations (10^7 – 10^9 bacteria/cc) the barrel of the pipette may appear (to the unaided eye) to be free of colonies except that at the margin between the inocu-

lated and control nutrient areas a clearly defined ring occurs. Seeing this ring, the observer will recognize that individual colonies are not visible, because the colonies are microscopic or because the nutrient has become completely overgrown indicating, thereby, a very high bacteria concentration. At the concentration in the $10^4 - 10^6$ bacteria/cc range proportional densities of colonies per unit length of pipette will be seen.

Lighting means **19, 21** are located below base **13** and are directed so as to provide maximal oblique illumination of the portion of pipette **18** being examined. Although a single source of light may be employed, it is preferred to use a pair of lamps directed so that the lighting patterns of the two lamps crosses along the axis of pipette **18**.

The placement of lamps **19, 21** relative to pipette **18** is such that rays of light emanating from these lamps and reaching the surface of pipette **18** intersect therewith such that these rays are not reflected to the lens **11** (and thereby to the eye of the observer). Also, with lamps **19, 21** disposed below base **13** there is no direct passage of light to lens **11** and the system functions essentially as a dark field viewer with the colonies receiving and scattering light. It is this scattered light that reaches the eye of the observer via lens **11** making the colonies visible as spots brighter than the surrounding, less-illuminated background.

Thus, it is required that light from the light source must not pass directly to, or be reflected to, the eye of the viewer and the intensity of illumination (light flux) from the light source must be high enough that the light scattered by the colonies that reaches the eye of the observer is of greater intensity than the ambient light reflected from the surface of the pipette **18**. Any position of one or more light sources meeting these criteria may be employed.

Device **10** may be used for the optical determination of microbial colony population density in ordinary ambient light conditions and, in fact, the ambient light may serve to illuminate the standards on comparator card **14** with or without the aid of light from the light source(s).

The power required for lighting bulbs **19, 21** may be provided by batteries (not shown) or the bulbs may be connected to an AC/DC power source (not shown).

Although the utilization of device **10** for the viewing of pipettes has been used to illustrate this invention, nutrient-coated glass slides or nutrient-lined test tubes may also be viewed and compared in the same manner using suitable variations of clamping means **17**. In the case of pipettes and test tubes the angle of incidence α with the central axis thereof of all light rays reaching the sampling/culturing device should be greater than 45°.

Also, advantageously the mounting of sockets **22** and **23** on mounting block **24** may be such as to permit adjustment of the angle α.

The viewer-comparator device **30** of FIG. **3** is a somewhat larger unit providing non-magnified visual readout. In its simplest version, it consists of base **31**, light source **32** connected to base **31** via front wall **33**, vertical support bracket **34**, light shield **36** and comparator disc **37**.

During readout, the nutrient-lined pipette **38** is supported so as to provide angle $\alpha\alpha$ the central axis thereof having a value of greater than 45° for the light emanating from lamp **32** and impinging thereon. Cleat **39** locates the lower end of the pipette and the upper end rests against the horizontal member **41** of frame **34**.

Comparator disc **37** is essentially the same as comparator card **14** although the standards may be separated further permitting location of a slot therebetween to accommodate pipette **38** between any two standards for ease of comparison. The standards, of course, need not vary in steps of 10X the previous density, but may be in other multiples such as in increments of 5X the previous density.

The distance, which light shield **36** extends forward, is such as to prevent the passage of light rays directly from light source **32** to the eye of the observer. Elevating means (thumb screw **42**) is provided to enable adjustment of the position of pipette **38** (and device **30**) relative to the observer. The position of light bulb **32** is also adjustable to provide the requisite angle α.

Device **30** is of particular value in viewing large numbers of pipettes in rapid sequence as in the laboratory of a hospital due to the ease of positioning the pipettes for readout.

Device **10** can be made as a foldable unit much as, for example, foldable viewers for examining 35 mm transparencies are made.

The comparator card may be a photograph, a transparency or similar reproduction. In the case of a transparency it may be advantageous to use a ground glass plate as a backing for the transparency.

What I claim as new and desire to secure by Letters Patent of the United States is:

1. A viewer/comparator device for optically determining microbial colony population density on a transparent surface having an inoculated nutrient layer thereon comprising in combination:

a. an opaque base,

b. at least one light source,

c. means for mounting said light source on one side of said base,

d. means connected to said base for positioning a transparent tubular member so that at least a portion of the length thereof is located on the opposite side of said base from said light source in which position light rays from said light source must pass directly to said portion to impinge thereon at an angle of incidence of greater than 45° with the central axis of said tubular member,

e. means for enabling direct comparison by an observer between the appearance of said portion and reproductions of microbial growth at a plurality of representative intervals over a range of clinical interest, said comparison-enabling means being disposed adjacent the location available for the positioning of said portion and

f. a magnifying lens supported on said opposite side of said base being spaced from said portion and disposed for simultaneous viewing of said portion and said comparison-enabling means, said light source being so directed that light rays passing therefrom to said opposite side of said base cannot pass directly to, or be reflected to, said lens.

2. The viewer/comparator device of claim **1** wherein a pair of light sources are employed, said light sources being directed so that the patterns of illumination of

the two lamps cross each other at the portion of the tubular member being examined.

3. The viewer/comparator device of claim 1 wherein the attitude of the light source is adjustable.

4. The viewer/comparator device of claim 1 wherein the positioning means supports the tubular member at two spaced points.

5. The viewer/comparator device of claim 1 wherein the positioning means is a spring clamp.

6. The viewer/comparator device of claim 1 wherein the comparison-enabling means is a photograph.

* * * * *