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METHOD AND APPARATUS FOR THE ASSAY OF VIABLE MICROORGANISMS

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Fig. 1.

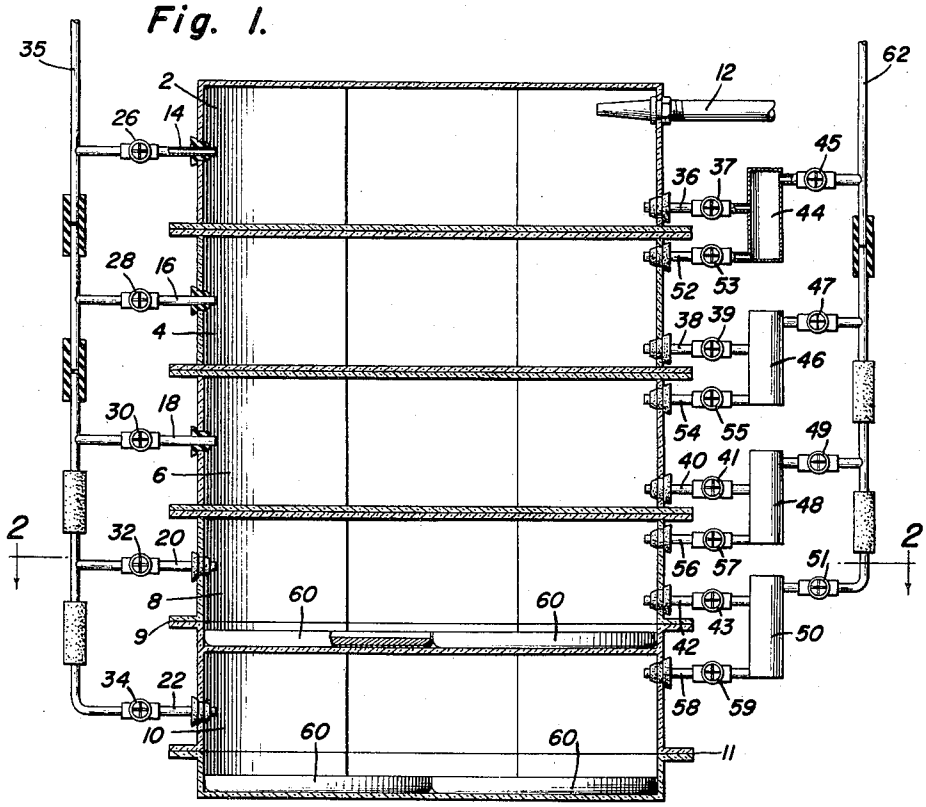
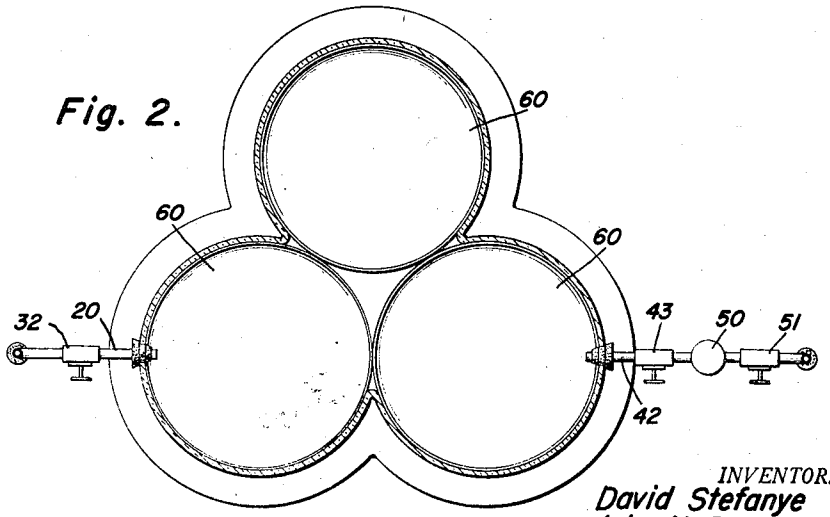


Fig. 2.



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METHOD AND APPARATUS FOR THE ASSAY OF VIABLE MICROORGANISMS

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The invention described herein may be manufactured and used by or for the Government of the United States of America for governmental purposes without the payment to us of any royalty thereon.

This invention relates to an apparatus for diluting an aerosol to enable a count to be made of the viable organisms therein. More specifically, the invention relates to a mechanism for preparing and diluting an aerosol by a known amount and of depositing the remaining or a proportion of the remaining organisms on a nutrient media for culturing and counting.

The established method for determining the number of viable organisms in a liquid is to introduce a measured amount of liquid into a large amount of water or other diluent and shake the resulting mixture. A sample is then withdrawn, rediluted and reshaken until a final degree of dilution is obtained such that a given volume of liquid may be plated out on a suitable media in a Petri dish and incubated. The resulting colonies are then counted and the original concentration determined from the dilution ratio. This method of determining the presence and number of viable organisms in a liquid has some disadvantages. It is quite slow, requiring much mixing, and the repeated sampling and redilution may be dangerous when toxic organisms are handled.

The present invention avoids these difficulties by making an aerosol of a given quantity of liquid and rapidly diluting the same before any appreciable settling takes place. This procedure eliminates the mixing and shaking problem inherent in dilution in the liquid state. Moreover, the aerosol is continuously inside of the apparatus during the entire determination so that no contamination can take place and the plating out on Petri dishes is automatically taken care of. Moreover, by utilizing a suitable atomizer or nebulizer, aerosol particle size approaches the dimensions of a single microorganism, thus causing chains and clusters of cells to separate, whereupon a more exact count is realized.

In Figure 1, beginning at the top, the five sections of the apparatus are shown in descending order at **2**, **4**, **6**, **8** and **10**. Atomizer inlet **12** in section **2** provides for the admission of the aerosol into the upper chamber. Stopcocks **26**, **28**, **30**, **32** and **34** serve to connect the respective sections with line **35** for admitting a sterile gas to the system. In a similar manner, the respective compartments **2**, **4**, **6** and **8** are fitted with outlet connections **36**, **38**, **40** and **42**, which are equipped with stopcocks **37**, **39**, **41** and **43** respectively. Corresponding inlet connections **52**, **54**, **56** and **58**, together with stopcocks **53**, **55**, **57** and **59**, are fitted to chambers **4**, **6**, **8** and **10**. These inlet and outlet connections are joined to sub-chambers **44**, **46**, **48** and **50**, the outlet from one chamber and inlet to the next lower chamber being connected to the same sub-chamber. By means of these several connections it is possible for an aerosol to be passed from one compartment to another via the sub-chambers and connections therebetween. The sub-chambers are vented into line **62** by means of stopcocks **45**, **47**, **49** and **51**.

The respective chambers and sub-chambers may be of any reasonable size, but for convenience in calculations the following sizes were used:

	Vol. in ml.
Chamber:	
2	990+.5
4	980+.5
6	980+.5
8	980+.5
10	1,000
Sub-chamber:	
44	10+.1
46	10+.1
48	10+.1
50	110+.1

By choosing these sizes, the following volumetric relationships obtain:

Vol. 2+vol. 44=1,000 ml. and vol. 44=a 10^2 dilution.

Vol. 4+vol. 44+vol. 46=1,000 ml. and vol. 46=a 10^2 dilution.

Vol. 6+vol. 46+vol. 48=1,000 ml. and vol. 48=a 10^2 dilution.

Vol. 8+vol. 48+vol. 50=1,000 ml. and vol. 50=a 10^1 dilution.

The apparatus may be constructed of glass, metal, ceramics or plastics, the only requirements being that it shall be airtight, capable of sustaining a substantial vacuum and must be capable of being sterilized by steam, heat, chemicals or gases. In addition, the walls should be smooth and not toxic to the organisms tested. In the preferred form the apparatus is made of glass having ground joints at **9** and **11**.

The transverse shape of the present apparatus is trifoliate, although a circular or other cross section could also be used. The section shown was chosen since it permits the close fitting of three Petri dishes as shown in Fig. 2. With this design, almost all of the aerosol settling out will fall on one or another of the dishes.

The two lower sections **8** and **10** are fitted with transverse sealed joints **9** and **11**, thereby permitting the sections to be opened for the placing or removing of the Petri dishes.

In operation, the apparatus is assembled and sterilized with steam, with all stopcocks open. The apparatus is then cooled to about 37° C. and the Petri dishes with their nutrient media are inserted in chambers **8** and **10**. The purpose of having plates in both of the lower chambers is to obtain an adjacent count differing by a power of 10. Stopcocks **26**, **28**, **30**, **32** and **34** are closed and the entire apparatus is evacuated through line **62** after which all stopcocks are closed. A measured amount of liquid containing viable organisms to be counted is now introduced into chamber **2** through the spray nozzle **12** as a finely divided aerosol. Sterile air or inert gas is admitted through stopcock **26** to bring chamber **2** to substantially atmospheric pressure. A sample of the aerosol is now taken into sub-chamber **44** by opening stopcock **37**. Subchamber **44**, being under a vacuum, will fill promptly from chamber **2**, the latter being at atmospheric pressure. This latter sample now contains $1/100$ of the organisms originally present in chamber **2**. Stopcocks **26** and **37** are closed and **53** opened, thereby transferring most of the sample in sub-chamber **44** to chamber **4**. The total volume of the selected sample of aerosol is now volume 4+volume 44. Stopcock **28** is opened to bring about equilibrium with sterile air or gas as before. Stopcock **39** is then opened to transfer part of the sample to sub-chamber **46**. The total volume occupied by the selected aerosol sample is now vol. 4+vol. 44+vol. 46,

This total volume has already been defined as 1,000 ml. and since vol. 46 is 10 ml., there is again a dilution of 100 to 1 or a total of 10,000 to 1 (10^4) between vol. 46 and the initial aerosol volume.

Stopcocks **28** and **39** are now closed and **55** opened, thereby largely transferring the aerosol into chamber **6**. Stopcock **30** is opened for pressure equilibrium as before and the sample partially transferred in succession to sub-chamber **48**, then chamber **8**, sub-chamber **50**, and chamber **10**. The stabilization of pressures through stopcocks **26, 28, 30, 32** and **34** is not essential to correct results, but it assists the movement of aerosol from chamber to chamber by maintaining the required pressure differential. The manipulation of stopcocks should be as indicated. Stopcocks **26, 28, 30, 32** and **34** are closed in order as indicated to prevent line **35** from being contaminated from the content of the upper aerosol chambers. In each transfer of aerosol there is a specific dilution of 100 to 1 and in the last stage between chamber **8** and sub-chamber **50** there is a dilution of 10 to 1. This gives a multi-stage dilution of 10^2, 10^2, 10^2 and 10^1 or a total of 10^7 to 1.

This dilution is sufficiently high so that when the organisms reach chamber **10** they are so sparse that upon settling out on Petri dishes **60** and incubated, the resulting colonies can be counted. This count, multiplied by the dilution ratio, gives the organism count in the original liquid sample with a high degree of accuracy.

The use of settling out in both chambers **8** and **10** in a 10 to 1 ratio provides a check against the accuracy of the findings of the lower chamber. In practice it is not feasible to wait for the settling out of every viable particle in chamber **10**. On the contrary, it is adequate to permit the greater number of particles to settle and then to remove the particles remaining after a specified time, by means of the exhaust system. In practice, a settling time of one-half hour results in an almost complete count of all viable particles. Since settling time varies for different size particles it is desirable to standardize the apparatus for different particle sizes. Thus, where particle size range of a given aerosol is known, this standardization permits of some economy of time in running the determination.

In operating the mechanism, the entire dilution procedure can be carried out in a matter of minutes and before any appreciable settling out takes place. After the multi-stage dilution has been carried out it will be noted that the several chambers are full of aerosol at the various concentrations obtained at that particular stage. Thus, the settling out that is allowed to take place in stage **8**, in nowise affects the subsequent dilution into stage **10**. In order to preclude extensive settling in stages **2, 4** and **6**, the aerosol contents of these stages may be exhausted through **62** as soon as the aerosol dilution has been carried beyond that particular stage. This reduces somewhat the cleaning necessary in the various stages.

We claim:

1. A dilution apparatus comprising a series of sealed main volumetric stages, intermediate smaller substages connected in series with said main stages and having valved inlet and outlet connections between stages, each substage having additional valved outlet connections into a common conduit to permit evacuation of the apparatus.

2. A method of diluting and culturing an aerosol of viable organisms which comprises enclosing a volume of the aerosol, withdrawing a fractional decimal volume of the original volume, enlarging said smaller volume, withdrawing a further fractional decimal volume and repeating this process in subsequent stages until the aerosol is sufficiently dilute, allowing the diluted aerosol to settle out on a nutrient media, culturing and counting the resulting colonies and multiplying this value by the decimal dilution factor to secure the number of organisms in the original volume.

3. Apparatus in accordance with claim 1, wherein an additional vent is fitted to each main stage to permit direct admission of gas to said stage.

4. An apparatus in accordance with claim 1, wherein the last main stage is fitted with Petri dishes to enable the culturing of any organisms settling out thereon.

5. An apparatus in accordance with claim 4, wherein the apparatus is made of glass in a trifoliate section.

6. An apparatus in accordance with claim 1, wherein the first stage is fitted with an atomizer for introducing an aerosol into the stage.

7. An apparatus in accordance with claim 1, wherein the volumetric ratios of the various stages and substages are such that the ratio of the volume of the first substage to the sum of the volumes of the first stage and substage is a positive power of 10 and the ratio of the volume of subsequent substages to the sum of the volumes of the substage in question plus the previous stage and substage is a positive power of 10.

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