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Sharpe

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[54] **APPARATUS FOR PERFORMING BACTERIOLOGICAL TESTS AUTOMATICALLY**

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[21] Appl. No.: **371,649**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 190,871, Oct. 20, 1971, abandoned.

[30] **Foreign Application Priority Data**

Oct. 29, 1970 Great Britain 51377/70

[52] **U.S. Cl.** **195/139,** 195/103.5

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

[58] **Field of Search** 195/103.5, 127, 139

[56] **References Cited**

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[57] **ABSTRACT**

An apparatus for bacteriological sampling in which Petri dishes are automatically provided with the ingredients for performing bacteriological colony counts. Sequentially diluted samples can be prepared by the apparatus.

7 Claims, 8 Drawing Figures

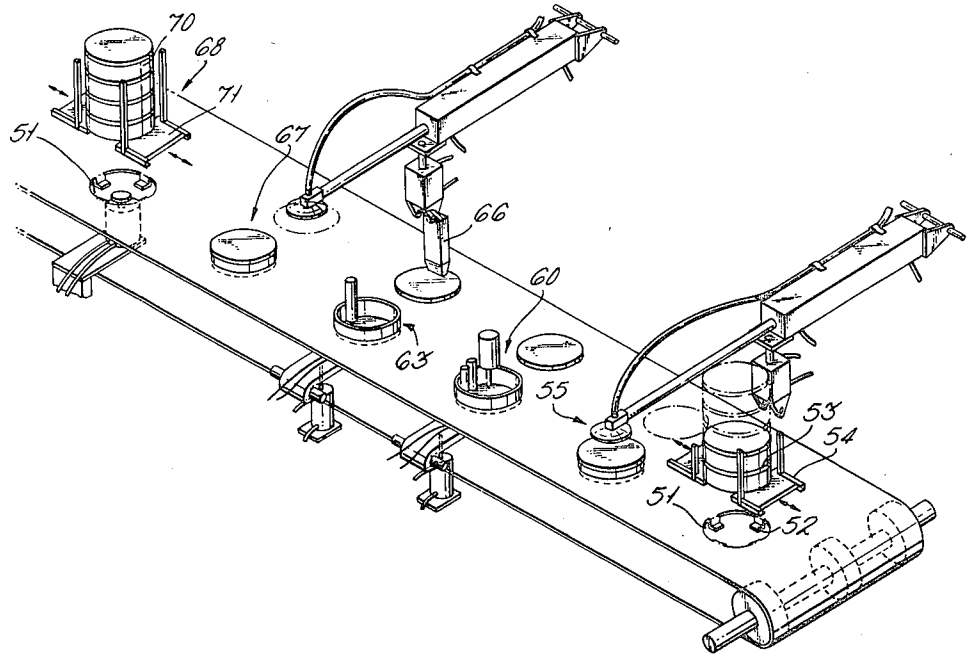
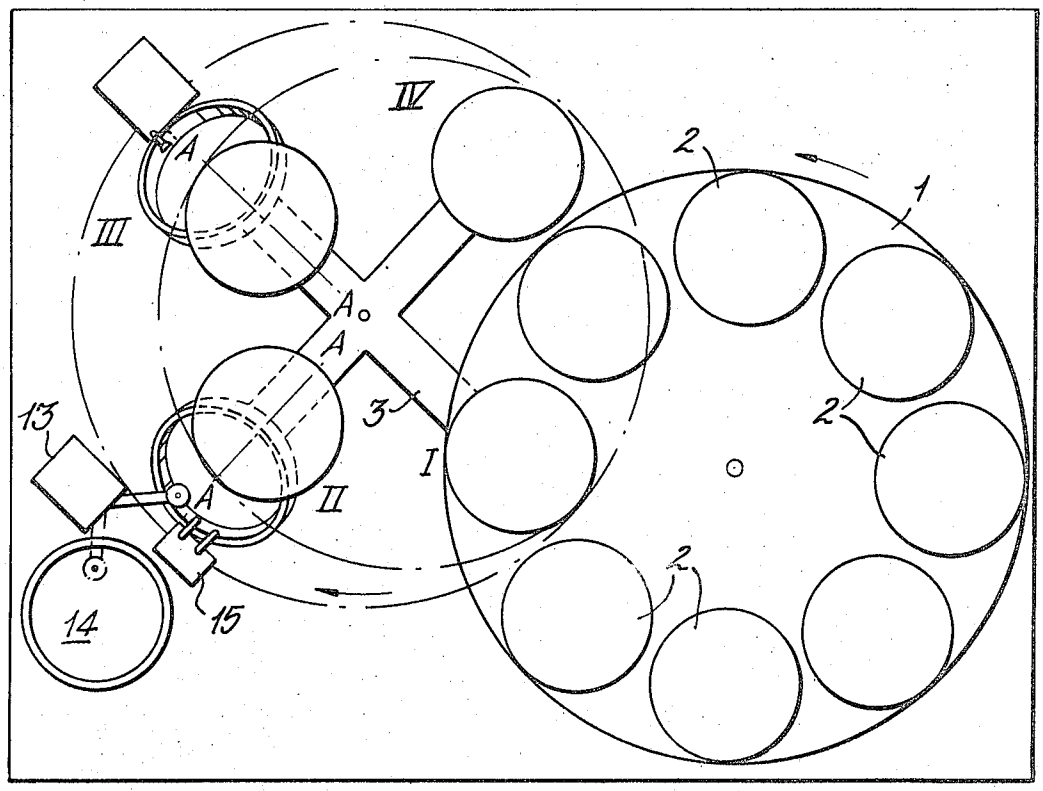


FIG. 1.



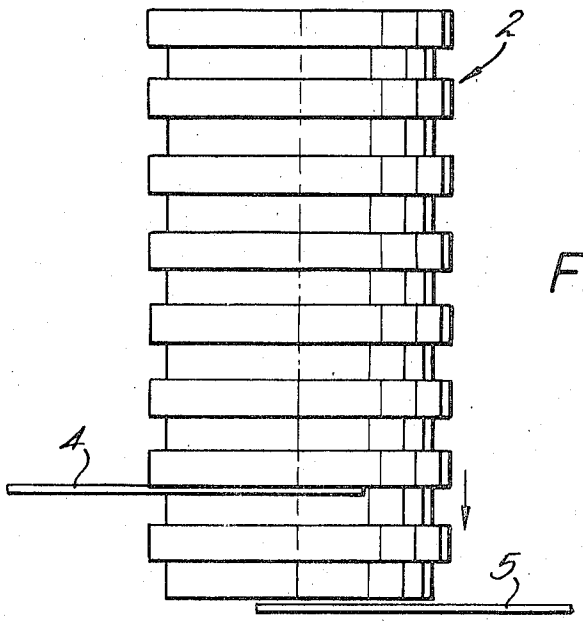


FIG. 2.

FIG. 3.

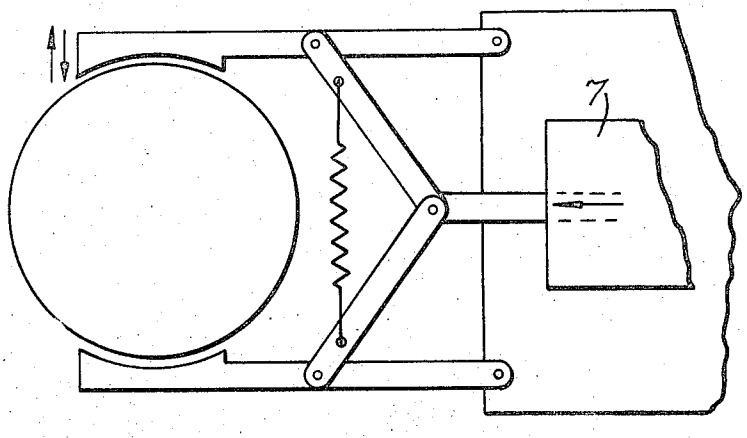


FIG. 4.

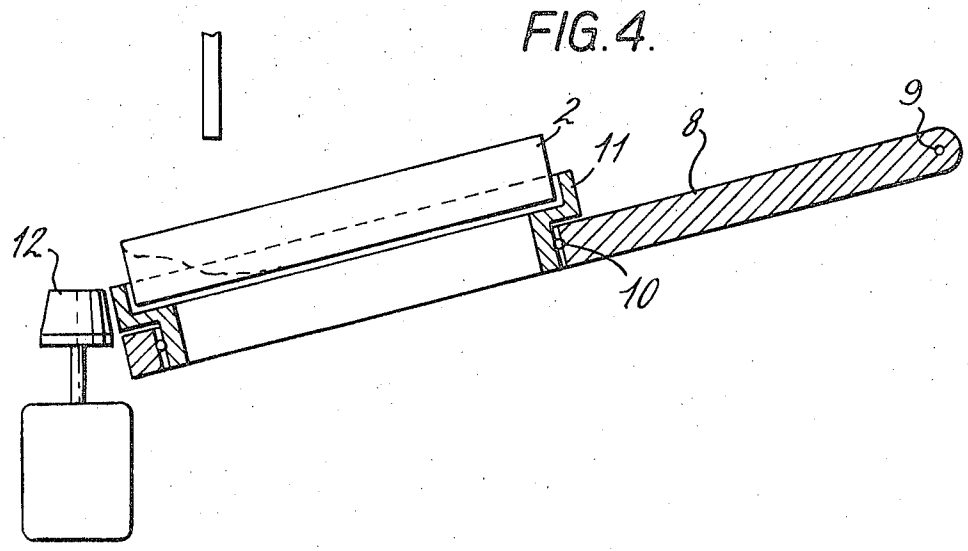
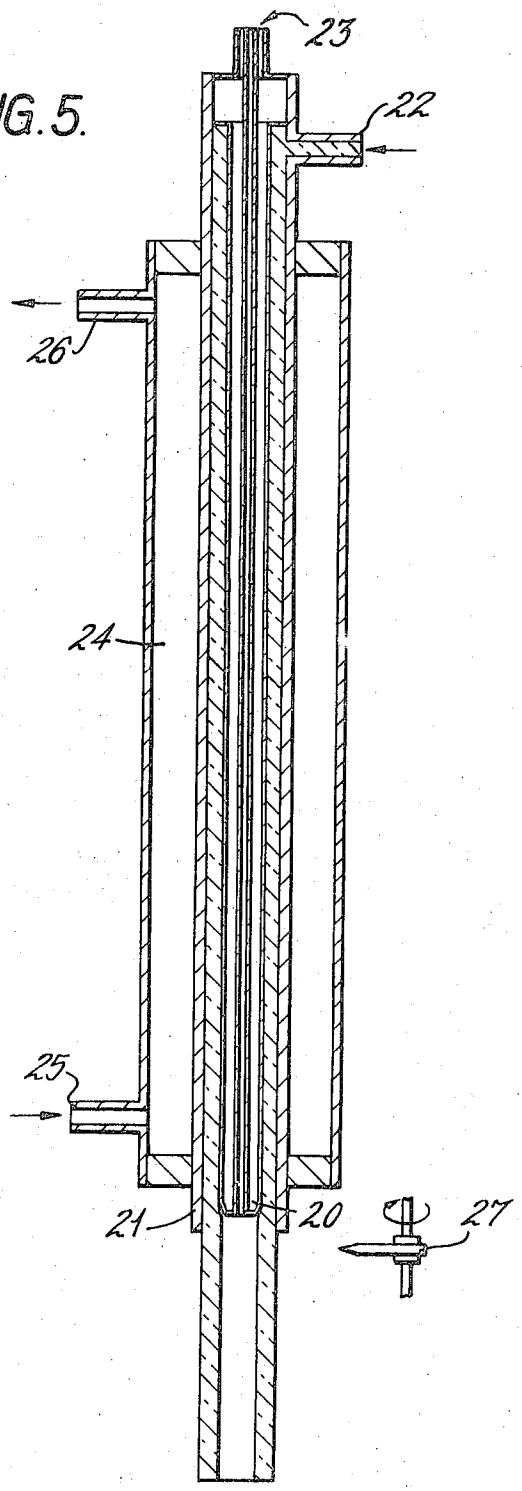


FIG. 5.



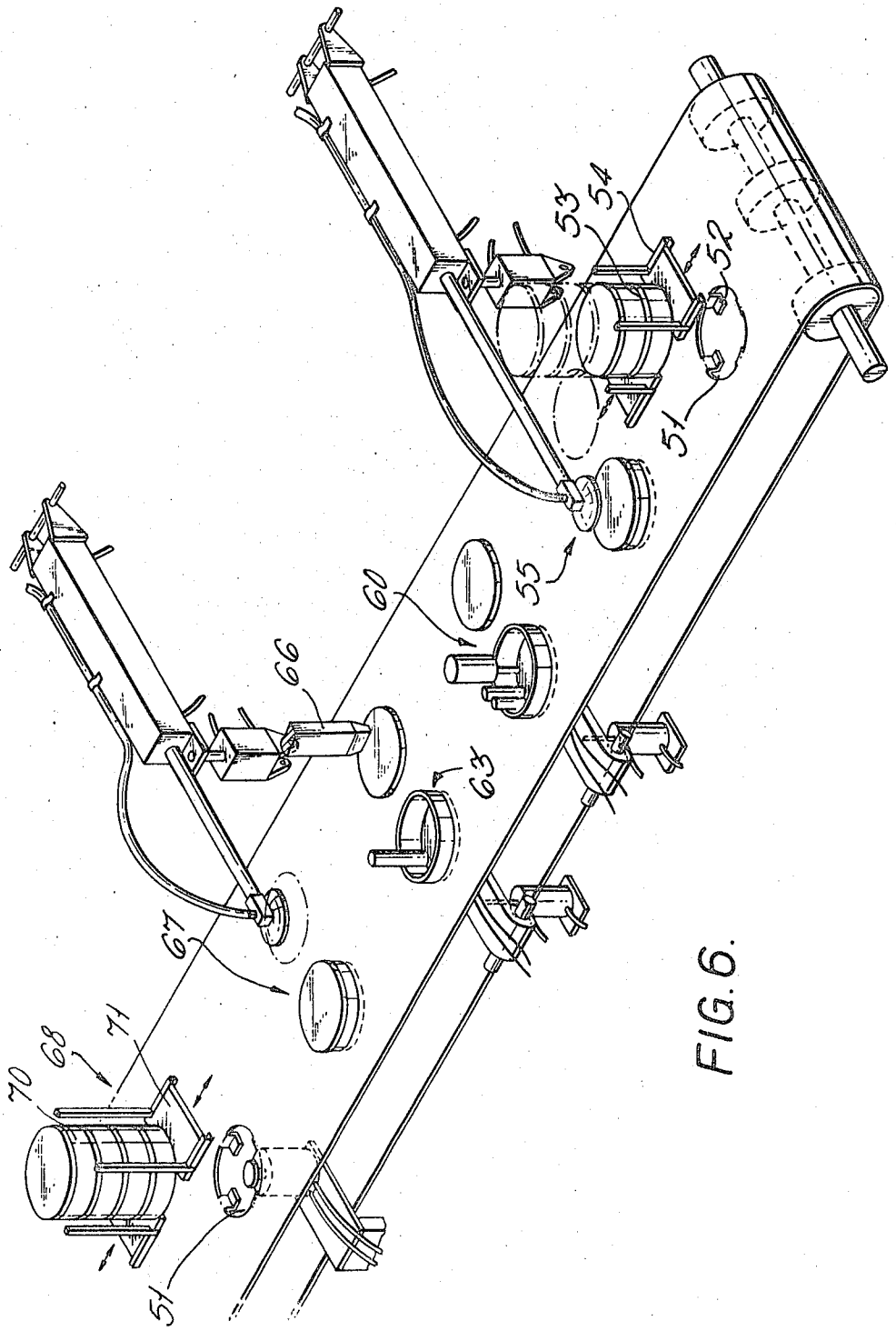


FIG. 6.

FIG. 7.

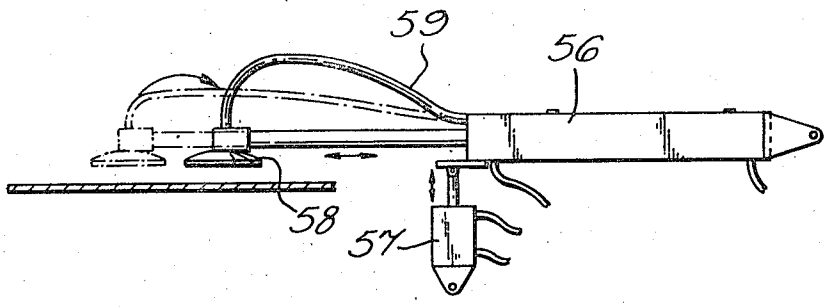
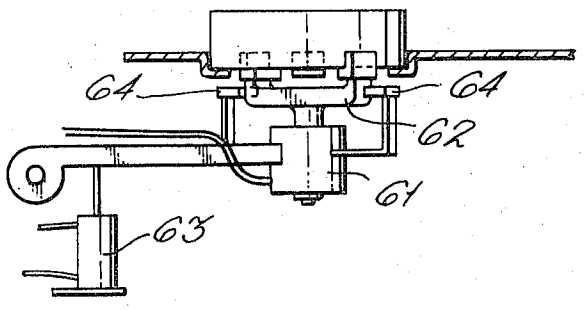


FIG. 8.



APPARATUS FOR PERFORMING BACTERIOLOGICAL TESTS AUTOMATICALLY

This is a continuation-in-part of our copending application Ser. No. 190,871 filed Oct. 20, 1971, now abandoned.

The present invention is concerned with methods and apparatus for performing bacteriological tests automatically.

In one of the commonest techniques of bacteriology, a technician is required to prepare a set of labelled Petri dishes filled with a nutritive jelly (usually based on agar-agar) and inoculated with sequential dilutions of a bacterial suspension. During incubation at a suitable temperature, each viable bacterium multiplies many times and gives rise to a visible colony in the jelly. From the number of colonies in the jelly and the dilution factor involved in making the inoculum for the dish, the number of viable bacteria in the original sample can be calculated.

The work involved in such a bacteriological test can be divided into two parts:

Firstly, background work which may be carried out by non-scientific staff, involving the preparation of the various reagents or media used in the test, dispensing these into bottles and sterilising them, cleaning and sterilising the various items of equipment such as bottles, Petri dishes and pipettes (if re-usable ones are used), and distributing these; and secondly, technical work which is the use of these already prepared items for the bacteriological test. In the second part, typically, a technician may make up to eight sequential 1/10 dilutions of the initial bacterial suspension by pipetting 1.0 ml successively through eight bottles each containing 9.0 ml of diluent, and inoculate 1.0 ml of each dilution into Petri dishes either singly or in duplicate. To each Petri dish is added melted jelly, into which the inoculum is thoroughly mixed by suitable movements of the dishes on a flat bench. After the jelly sets the dishes are incubated and eventually a count of colonies is made. At some stage in this process, usually at the beginning, all Petri dishes are labelled with the sample number and dilution number to guard against the possibility of their being mixed up before the count has been made. This labelling is time consuming.

In many instances, specific reagents which will inhibit or promote the growth of certain bacteria may be added to the gelling material in order to allow only certain species of bacteria to be counted by this method. In other instances specific reagents may be added which alter in some recognisable way the area around colonies of certain species, for example by a change in colour or transparency.

If desired Petri dishes containing different types of jelly may be routinely used by a technician for each sample, all sets of dishes being inoculated from the same set of dilutions. For example, on a particular sample, tests for total numbers of viable bacteria, and *Coliform* and *Staphylococcus aureus* bacteria may be made, each test requiring a jelly of different composition.

It is of the greatest importance in bacteriological testing that all equipment and materials are sterilised before use, otherwise considerable error may be introduced. It is equally important that at each dilution stage a fresh sterile pipette is used to prevent contamination of the lower concentration by bacteria adsorbed on the surface of the pipette in the high concentrations.

The invention is concerned with the provision of an automatic bacteriological system taking into account these requirements.

Accordingly the invention provides a bacteriological sampling apparatus comprising a media dish store,

a sample and diluent supply station,

a gelling agent supply station,

a discharge station, and

a media dish transport system for transporting each media dish from said store and to each of the stations in turn, the sample and diluent supply station comprising a rotary dish carrier for rotating a dish when at said station to mix liquid in said dish, a diluent supply means, and a bacteriological sample pipetting means which is arranged to supply a sample to each dish in turn and after mixing to withdraw from the dish under sterile conditions a portion of mixed diluted sample and to hold said portion for supply to a following dish, and the gelling agent supply station comprising gelling agent supply means, and a rotary dish carrier for rotating the dish to mix diluted sample with gelling agent.

Preferably the sample supply means comprises a device which is arranged to extrude a tubular nozzle before use, for use as a pipette in transferring sample from one dish to the next, and to discard said nozzle after use. Thus a fresh nozzle may be formed immediately before transferring a diluted sample from one dish to the next. This provides an automatic system where the risk of contamination in use is of a very low order. As an alternative to forming the fresh pipette by extrusion, a used pipette may be sterilised for re-use or a new independently manufactured pipette may be supplied, however the in situ extrusion method is found to be particularly convenient.

Conveniently the apparatus comprises a store for agar, means for supplying a quantity of said agar to the gelling agent supply station to serve as a nutrient and jelly, and means for supplying a further quantity of said agar to the sample supply means for extrusion to form a nozzle.

By use of this apparatus the ingredients are mixed automatically in the dish rather than with the aid of various bottles and other equipment which would otherwise be necessary. This reduces considerably the amount of hand operations to be carried out by the technician thereby enabling a suitable mechanised system to be devised, resulting, inter alia in a higher through put.

Moreover by adding gelling agent, i.e., jelly at a separate station from sample and diluent, sequential dilutions can be performed automatically by providing a fresh pipette and withdrawing a portion of mixed diluted sample from the dish at the sample supply station after mixing, and holding this portion in the pipette until a following dish is in position to receive sample at the sample supply station.

In one particular form of the invention, for example the throughput of prepared dishes that could be achieved in an 8 hour working day was of the order of 2,000 and a saving of 70 percent of the total labour requirement for a bacteriological count was achieved. This output considerably exceeds the capabilities of most bacteriologists and moreover there are considerable savings in use of ancillary equipment such as bottles which would need to be uncapped, washed, refilled, recapped etc. in a hand operation.

The media dish transport system may be a rotary system of radial arm carriers and this has been found to be the most convenient form of operation in practice. However another useful method is to use an in-line conveyor system operated for example by a form of belt conveyor, having cradles in its surface to carry the media dishes.

Two embodiments of the invention will now be described by way of example with reference to the accompanying diagrammatic drawings in which:

FIG. 1 shows a schematic plan view of an automatic bacteriological testing device;

FIG. 2 shows a side elevation of a stack of Petri dishes and a dish dispensing mechanism;

FIG. 3 shows a plan view of the dish dispensing mechanism;

FIG. 4 shows a sectioned side elevation on the line A—A of FIG. 1 indicating a dish mixing device;

FIG. 5 shows a sectioned side elevation of a Pipette extruding device.

FIG. 6 shows a schematic perspective view of the second embodiment, which is an in-line machine;

FIG. 7 shows a lid removing device; and

FIG. 8 shows a detail side elevation of a rotary dish carrier for the second embodiment.

The apparatus uses Petri dishes to hold liquids for a dilution process, and transfers liquid samples into each dish and from one dish to the next by means of a freshly extruded sterile agar-agar pipette, the manufacture of which is described herein.

Referring to FIG. 1, a rotary turntable 1 carries eight stacks of fresh sterile lidded Petri dishes 2. Each stack contains 20 dishes.

The rotary turntable 1 rotates in the direction of the arrow shown, to overlap with a rotary carrier 3. Rotary carrier 3 has four complex arm systems each of which is arranged to carry a Petri dish and its lid, the carrier being rotatable by a series of indexing movements to bring the arm systems in turn to each of four stations so that on each arm system the various stages of the process can be carried our progressively.

At the first station I a Petri dish and lid is received from the rotary turntable 1 by operation of a dispensing mechanism, shown in FIGS. 2 and 3. The dispensing mechanism comprises two pairs of claws 4 and 5 each of which is as shown in plan view in FIG. 3. Each pair of claws is operated by a mechanical linkage 6 and a solenoid 7 via which the claws can be moved into and out of gripping relationship with the stack. To dispense a Petri dish from the bottom of a stack the lower pair of claws 5 is released to allow a dish to drop onto the carrier system 3 while the upper pair of claws 4 holds the remainder of the stack. Actuation of the two pairs of claws is then reversed so that the whole of the stack falls to a position where it is held by the claws 5.

A Petri dish and lid will now be on the rotary carrier at station I. The rotary carrier then transports this Petri dish and lid by an indexing movement to station II and the next carrier arm system is positioned to receive a Petri dish. During movement from station I to station II by operation of various parts of the appropriate arm system of the rotary carrier, the lid is removed from the dish and displaced so that the dish can receive a sample.

Each complex arm system comprises a lower arm 8, shown in FIG. 4, which is pivoted about a fulcrum 9 near the centre of the rotary carrier. The arm 8 carries within a bearing 10 a rotary sub-carrier assembly 11 within which the Petri dish is carried. During movement of the rotary carrier to station 2 by virtue of a camming arrangement (not shown) the rotary arm 8 pivots from the horizontal down to a position where it is inclined at an angle to the horizontal and where, when it reaches the station II, the carrier engages with a rotary rubber driving wheel 12. Rotation of the driving wheel 12 causes the sub-carrier 11 and dish to rotate whereby any liquid in the Petri dish is mixed.

While the arm 8 moves downwards to its inclined position at station II, a second arm (not shown in detail) maintains the lid at its original horizontal position whereby the Petri dish lid is removed, and in addition, via a camming operation not shown, causes the lid to move radially inwards so that the upper part of the Petri dish is exposed to enable samples etc. to be dispensed thereinto.

At station II a pipette dispensing system (in accordance with the invention and to be described later) is located for the transfer of a measured quantity of a sample from a sample pot 14 into the Petri dish at station II, and thereafter to transfer dilute samples from one dish to the next following one. At the same time a nozzle from a diluent delivery system 15 is arranged to supply a measured quantity of diluent into the Petri dish. (Two nozzles in figure for different diluents.) While sample and diluent are being delivered into the dish, the dish is rotated via the driving wheel 12 shown in FIG. 4.

Following the mixing operation a fresh pipette withdraws a proportion of the dilute sample from the dish. Thereafter each following dish is supplied with the residual dilute sample then in the pipette and is further diluted after which a fresh pipette withdraws a quantity of that diluted sample, so providing a series of successively diluted samples.

Subsequent to completion of delivery and mixing of sample and diluent, the rotary carrier 3 is rotated to bring the filled Petri dish to the next station, station III. At station III a similar rotary mixing takes place while an agar delivery system 16 feeds a suitable quantity of melted agar into the dish.

The rotary carrier 3 is then indexed round to bring the Petri dish at station III to station IV. During movement from station III to station IV movements of the carrier arm system take place in reverse to those between stations I and II to bring the carrier arm 8 back to the horizontal and the lid carrier radially outwards so that at station IV the Petri dish is horizontal once more and has its lid in position. Subsequently at station IV by means not shown the dish is labelled and moved from the carrier mechanism and added to a stack of similarly prepared dishes. The empty carrier arm system is then indexed round to station I to receive a further dish.

The automatic pipette dispensing device at station II and as shown in FIG. 5 will now be specifically described.

The bore of an extruder is constituted by the passageway between concentric tubes 20 and 21. An inlet 22 to this passageway is connected to a supply of agar-agar so enabling a length of agar tubing to be extruded to form a pipette. The inner tube 20 is connected via an inlet 23 to an air pump which enables negative and positive pressure to be applied in turn to the interior of the pipette so formed, for drawing in and ejecting a sample.

The outer tube **21** is surrounded by a water jacket **24** which enables water to be pumped via an inlet **25** and an outlet **26** through the system to cool the agar as it passes through the nozzle, thus causing it to set before it appears at the outlet of the extruder. A cutter **27** is provided for cutting off a used portion of agar tubing.

In operation molten agar-agar is pumped into the nozzle via the inlet **22** and subsequently solidifies and is extruded into the shape determined by the inner and outer tubes **20** and **21.** A convenient cross section for the bore of the pipette so formed is clover leaf since this relatively large cross sectional area allows a large volume of liquid to be retained by the pipette with the minimum tendency to drip. Having extruded a suitable length of agar tubing to form a pipette, the whole device is then mechanically moved so as to dip the end of the pipette into the sample pot or a dish as shown in FIG. **1,** and suction is applied to draw a measured quantity of the sample into the bore of the pipette, care being taken to ensure that the sample does not come into contact with parts of the structure such as the tubes **20** or **21.** The device, while maintaining suction at **23,** is then raised and either moved across to a position above a Petri dish at station II or held until the next dish is in position, the suction is released and slight positive pressure applied at the inlet **23** so as to cause the sample to drop into the Petri dish **2.** The cutter **27** then severs and removes the used length of tubing to enable a fresh pipette to be extruded for use in the next operation.

Reference will now be made to FIGS. **6, 7** and **8** which show an alternative form of apparatus in which the apparatus is laid out to operate on an in-line system rather than a rotary carrier. In this case however the basic essentials of the invention namely a Petri dish store, a sample and diluent supply station, a gelling agent supply station and a dish transport station are as before in broad principle. Also present as before are the rotary dish carrier mixers and the arrangement of diluted sample transfer, prior to jelly supply, which enable sequential dilutions of the sample to be prepared in sequential dishes.

Referring specifically to FIG. **6,** an indexed belt conveyor **50** is provided with a series of apertures **51** of a diameter to accommodate a Petri dish and having four inwardly directed L-shaped lugs forming a cradle to carry a dish. These lugs may be formed by moulding during manufacture of the belt conveyor **51.**

A media dish store **53** at the commencement of the conveyor flight includes a dish dispensing mechanism **54** which is similar in operation to the dispenser mechanism shown and described with reference to FIG. **3.**

The belt conveyor by an indexing movement locates an aperture **51** under the dispensing mechanism **54** and a single dish and lid is dropped into that aperture.

A further index movement of the conveyor brings the dish to a de-lidding station **55.**

A de-lidding mechanism at the station **55** is shown in FIG. **7.** This consists of horizontal and vertical pneumatic cylinders **56** and **57** respectively for movement of a suction head **58** whose suction is produced via an air line **59.**

By operation of cylinders **56** and **57** the suction head is brought over and into contact with the Petri dish lid at the de-lidding station. Suction is then applied via air line **59** and the air cylinders **56** and **57** are activated to lift up the lid and deposit it at a point on the belt which is laterally alongside the dish (see FIG. **6** dotted position of lid).

By a further indexed movement of the belt **50,** the open dish is brought to a sample and diluent supply station **60.** At this station sample and diluent are supplied, and a portion of diluted sample withdrawn for further dilution in the next following dish. The mechanism for this is precisely the same as in the previous example using an extruded pipette of the type illustrated in FIG. **5** and will not therefore be described.

The dish mixing however is slightly different and is shown in FIG. **8** and will now be described with reference to this Figure.

As previously stated, the dish is carried on four inwardly directed lugs **52.** The rotary mixer comprises a motor **61** driving a four arm cradle **67.** A pneumatic cylinder **63** lifts the motor **61** so as to bring the cradle into contact with the dish with the four arms interposed between the lugs **51.** The cylinder **63** lifts the dish completely clear of the lugs **5** and rotation of the motor with the dish slightly tilted causes mixing of the dish contents.

Magnet pole pieces **64** are provided so that when the motor stops on completion of mixing it stops in a particular angular position to enable the cradle arms to pass through the lugs **52** without contact as the dish is lowered back onto the lugs.

The conveyor belt then conveys the dish containing sample and diluent to the next station, the nutrient supply station. At this station nutrient and jelly, generally agar, are supplied by a similar means to that in the first example, and the dish is rotated for mixing by a second rotary mixing mechanism of the type shown in FIG. **8.**

While gelling agent is being added to the dish at the gelling agent supply station, a code stamper **66** is arranged to stamp the lid with a code to label the sample and its level of dilution.

The dish, and labelled lid are then fed by the conveyor belt to a re-lidding station **67.** At this station a mechanism which is the same as that shown in FIG. **7** is used to replace the lid on the dish. The mechanism is identical to the de-lidding mechanism and only differs in that its operational sequence is reversed.

The dish and lid are then moved by the conveyor to a re-stacking station **68.** A pneumatic cylinder **69** lifts the dish off the conveyor belt and onto the bottom of a stack **70.** A re-stacking mechanism **71** similar to the mechanism **54** allows stacking from the bottom up to take place.

What is claimed is:

1. A bacteriological sampling apparatus comprising a media dish store,

a sample and diluent supply station,

a gelling agent supply station,

a discharge station, and

a media dish transport system for transporting each media dish from said store and to each of the stations in turn, the sample and diluent supply station comprising a rotary dish carrier for rotating a dish when at said station to mix liquid in said dish, a diluent supply means, and a bacteriological sample pipetting means which is arranged to supply a sample to each dish in turn and after mixing to withdraw from the dish under sterile conditions a portion of mixed diluted sample and hold said portion

for supply to a following dish, said sample pipetting means comprising a device which is arranged to extrude a tubular nozzle before use, means to sever said nozzle after use, and the gelling agent supply station comprising gelling agent supply means, and a rotary dish carrier for rotating the dish to mix diluted sample with gelling agent.

2. An apparatus according to claim **1** comprising a store for agar, means for supplying a quantity of said agar to the gelling agent supply station to serve as a gelling agent, and means for supplying a further quantity of said agar to the sample supply means for extrusion to form a nozzle.

3. An apparatus according to claim **1** in which said media dish transport system is a rotating carrier having a plurality of arms, each arm having means to releasably hold at least one media dish, and means to rotate the carrier by an indexing movement to the dish store to receive a dish therefrom then into juxtaposition with each of the supply stations in turn for supply and mixing of sample, diluent and gelling agent.

4. An apparatus according to claim **1** in which said media dish transport is on in-line conveyor having a plurality of cradles for carrying media dishes, means for moving the conveyor by an indexing movement to bring each cradle in turn to the dish store to receive a dish therefrom and then into juxtaposition with each of the supply stations in turn for supply and mixing of sample diluent and gelling agent.

5. An apparatus according to claim **1** wherein each media dish has a lid and the media dish transport system comprises lid removal means arranged for removal of the lid from each media dish in turn prior to supply and mixing of sample, diluent and gelling agent, and lid replacement means for replacement of the lid after supply and mixing of sample, diluent and gelling agent.

6. An apparatus according to claim **5** comprising labelling means for labelling said dish in dependence on the supply and mixing of sample, diluent and gelling agent therein.

7. An apparatus according to claim **6** in which said labelling means is arranged to apply the label to the dish lid during completion of ingredient supply and mixing with the dish.

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