United States Patent [19]

Chibata et al.

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[45] **Dec. 16, 1975**

[54] **METHOD OF CONTINUOUS AUTOMATIC INCUBATION OF BACTERIA AND DETERMINATION OF GROWTH THEREOF, AND APPARATUS THEREFOR**

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[21] Appl. No.: **399,309**

[30] **Foreign Application Priority Data**

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Sept. 27, 1972 Japan 47-96735

[52] **U.S. Cl.** **195/103.5;** 195/115; 195/139; 195/142

[51] **Int. Cl.**[2] **C12B 1/00**

[58] **Field of Search.** 195/103.5, 127, 139, 140–142, 195/115

[56] **References Cited**

UNITED STATES PATENTS

3,322,956 5/1967 Shah ..... 195/103.5 X

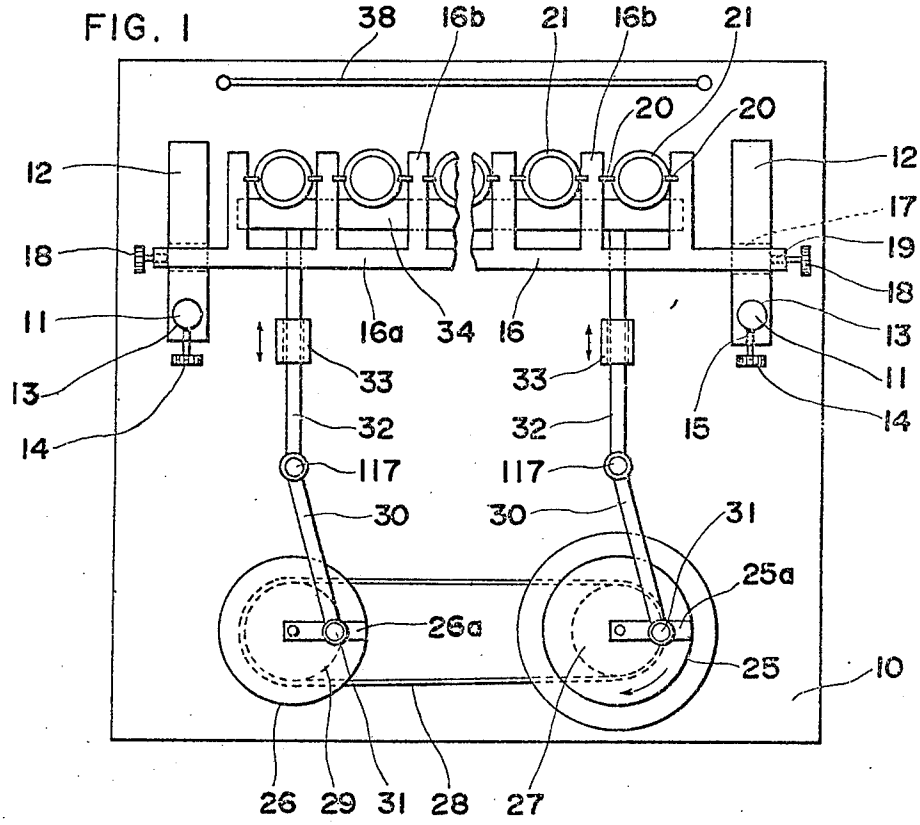
Primary Examiner—A. Louis Monacell
Assistant Examiner—R. B. Penland
Attorney, Agent, or Firm—Wenderoth, Lind & Ponack

[57] **ABSTRACT**

A method and a means for the culture of bacteria with or without shaking, in which supply of culture media and test material, the stages of inoculation, and periodic determination of the growth of bacteria are accomplished in a continuous, automatic process.

5 Claims, 16 Drawing Figures

FIG. 1


38
16b
21
16b
21
20
20
12
12
17
18
19
18
11
13
16a
34
16
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13
11
14
32
32
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25a
26a
25
10
26
29
31
28
27

FIG. 2

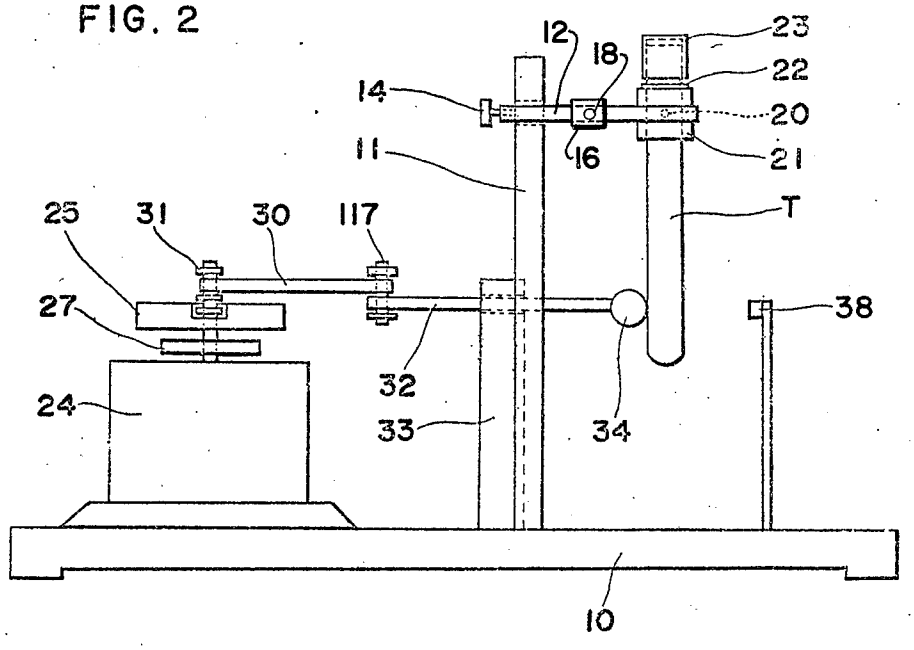

12
18
23
22
14
20
11
16
21
T
25
31
30
117
27
38
32
24
33
34
10

FIG. 3

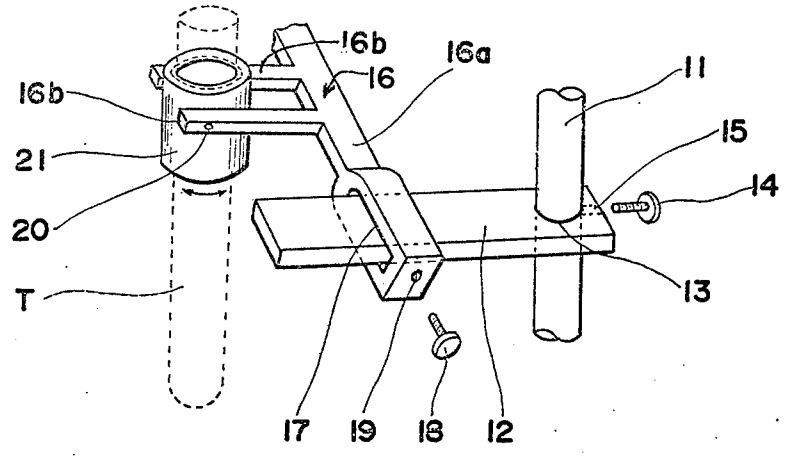


FIG. 5

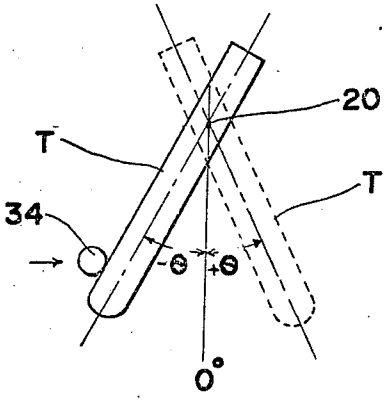


FIG. 6

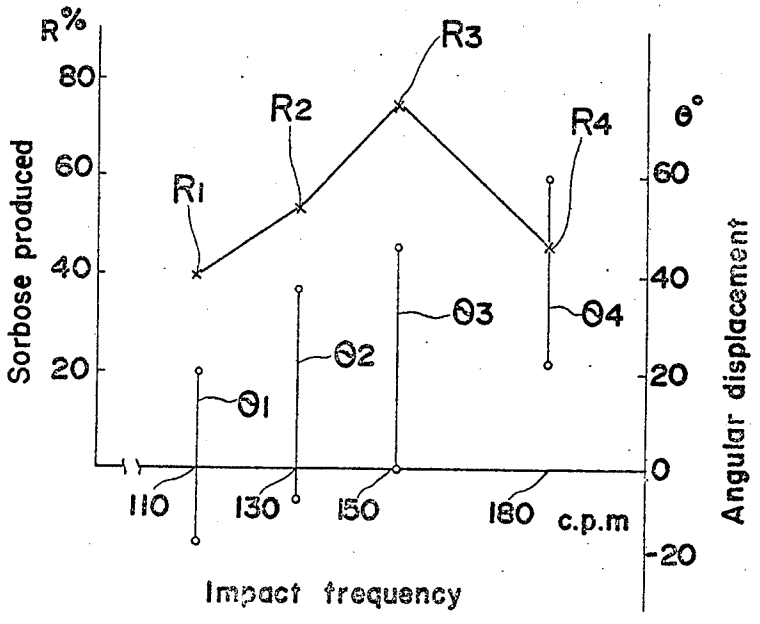
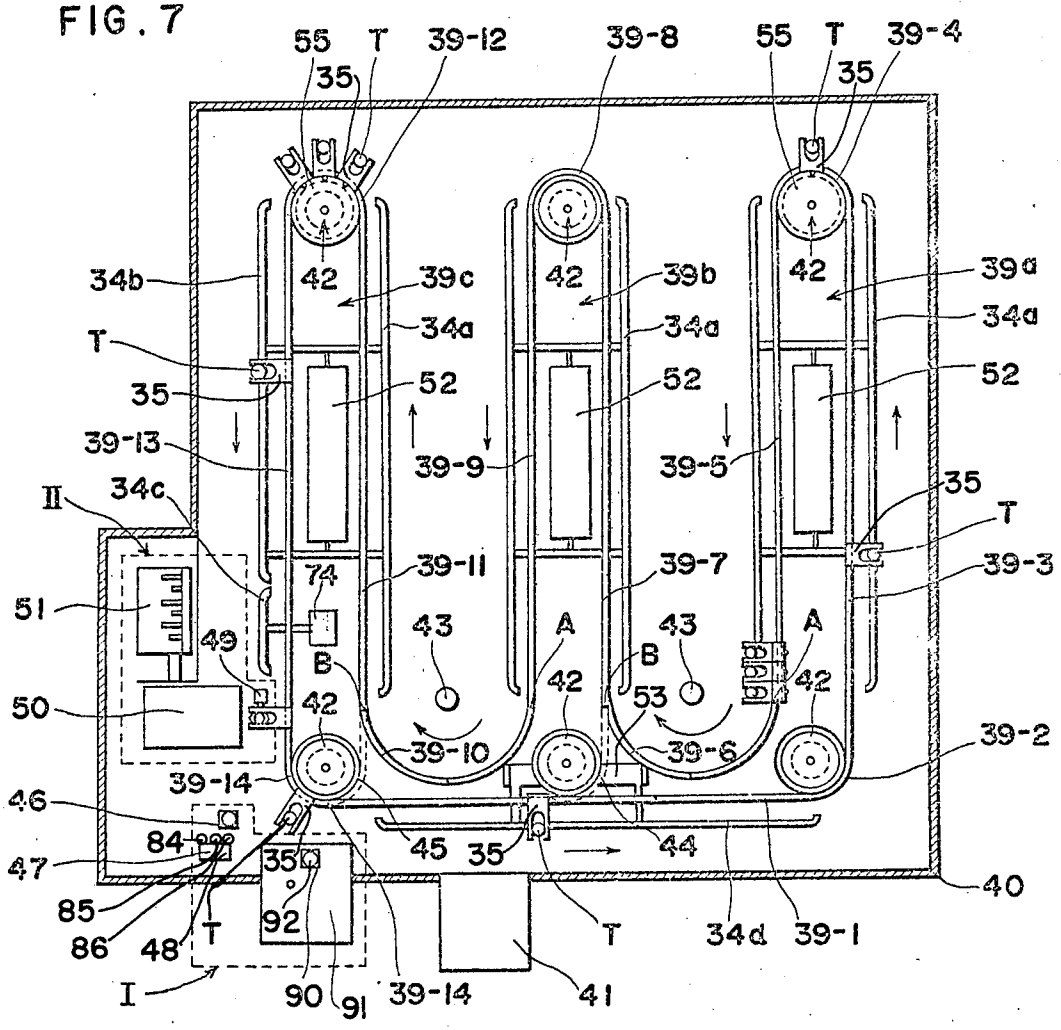


FIG. 7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T
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39-8
55
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39-4
35
35
42
42
42
34b
39c
39b
39a
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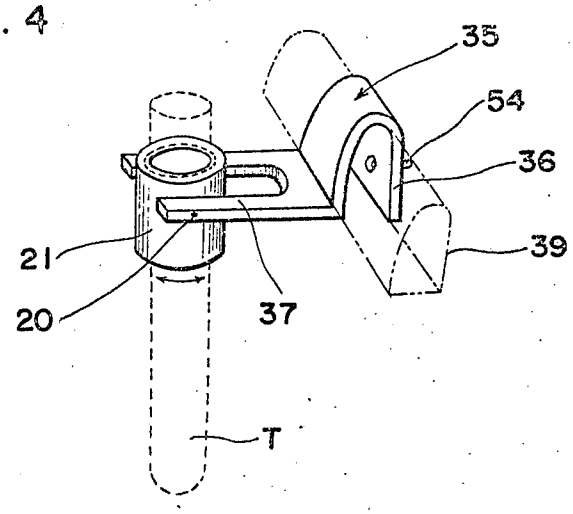
34a
T
52
52
52
35
39-13
39-9
39-5
II
34c
35
T
74
39-11
39-7
39-3
51
43
A
43
A
B
B
49
42
42
42
50
53
39-10
39-6
39-2
39-14
46
84
45
35
44
47
35
40
85
92
86
48
T
T
34d
39-1
I
90
91
39-14
41

FIG. 4


35
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21
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37
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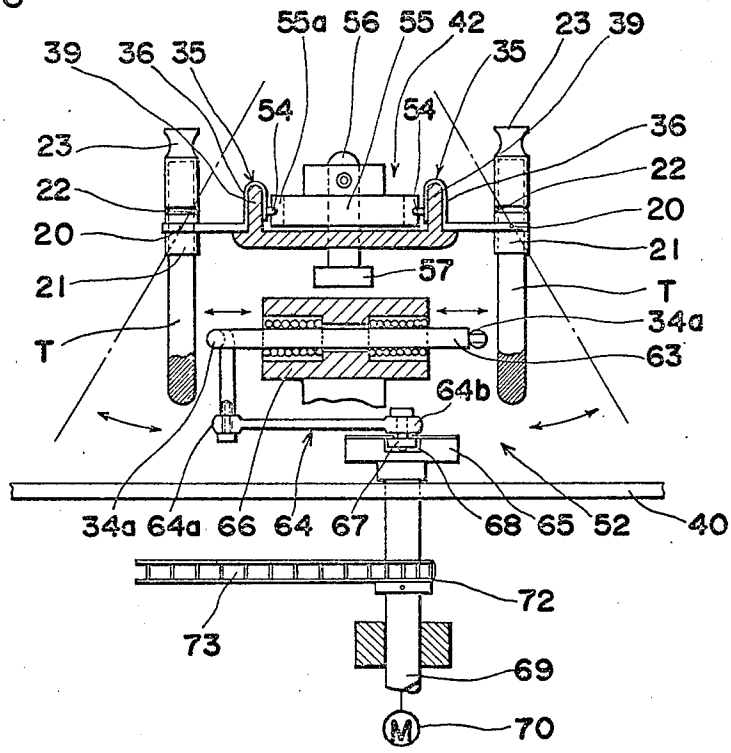
FIG. 8


55a 56 55 42
23 39
39 36 35
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21
21
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T
T
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64b
34a 64a 66 64 67
68 65 52
40
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73
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M
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FIG. 9

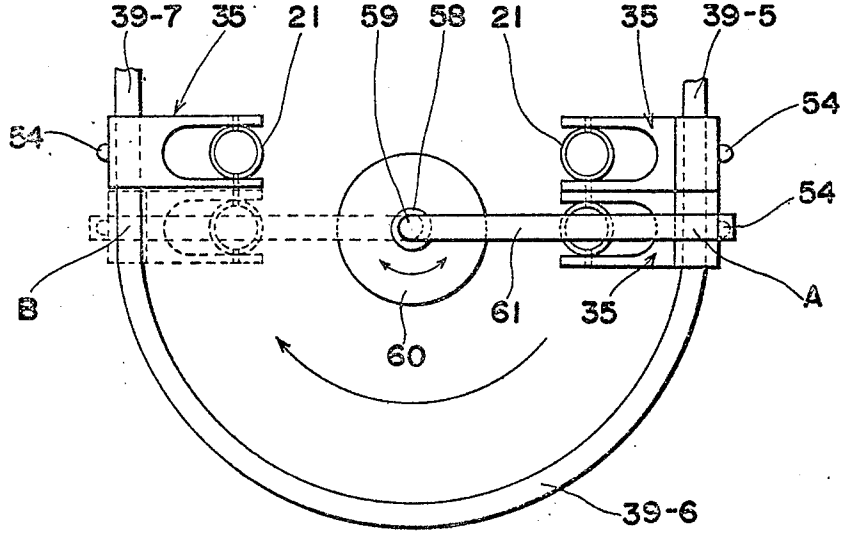
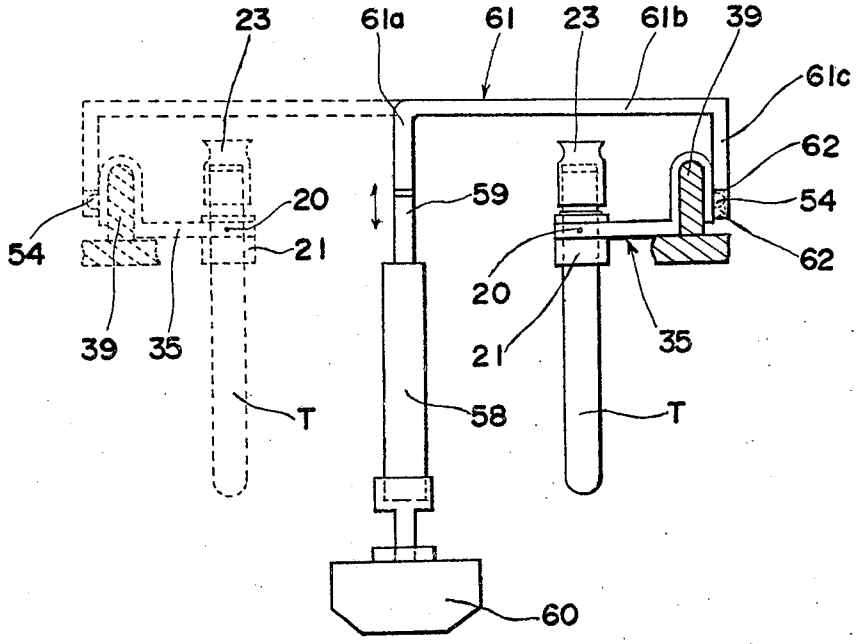

39-7 35 21 59 58 21 35 39-5
54
54
54
B
A
61
35
60
39-6

FIG. 10


23
61a
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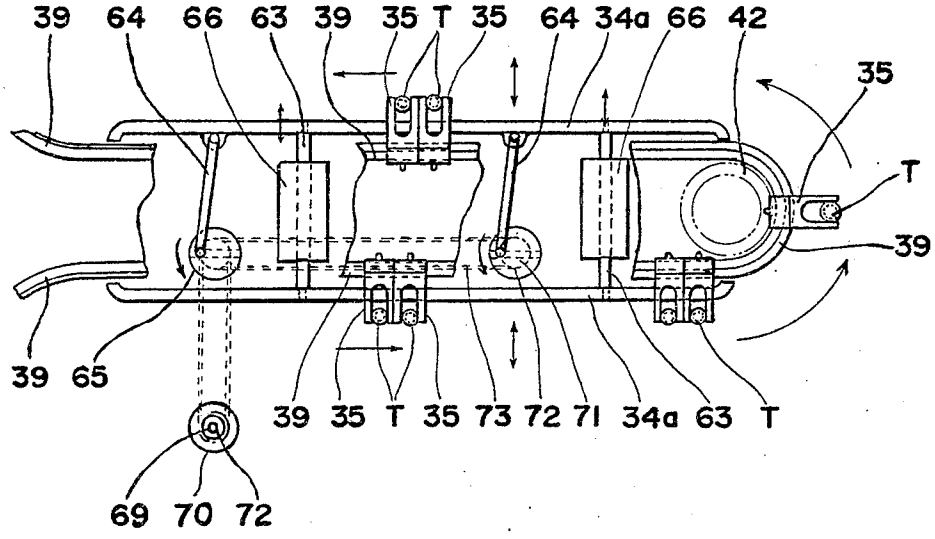
35
T
58
T
60

FIG. 11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34a
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42
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34a
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69
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72

FIG. 14

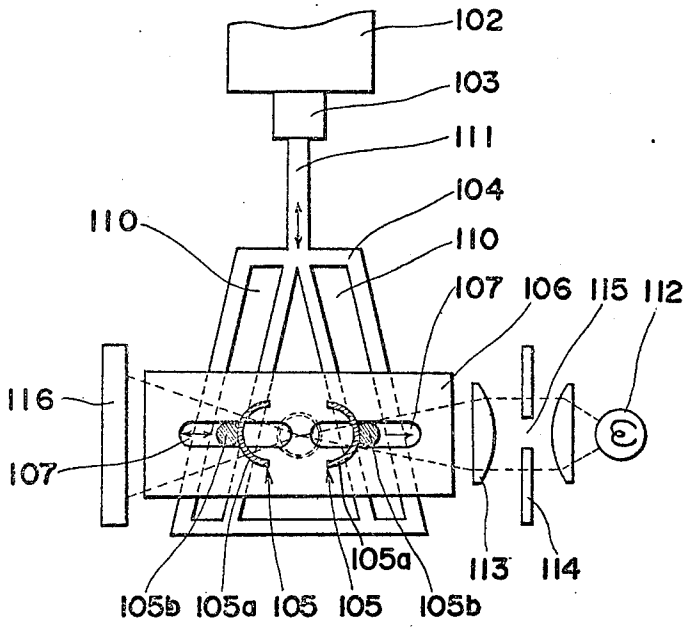


FIG. 15

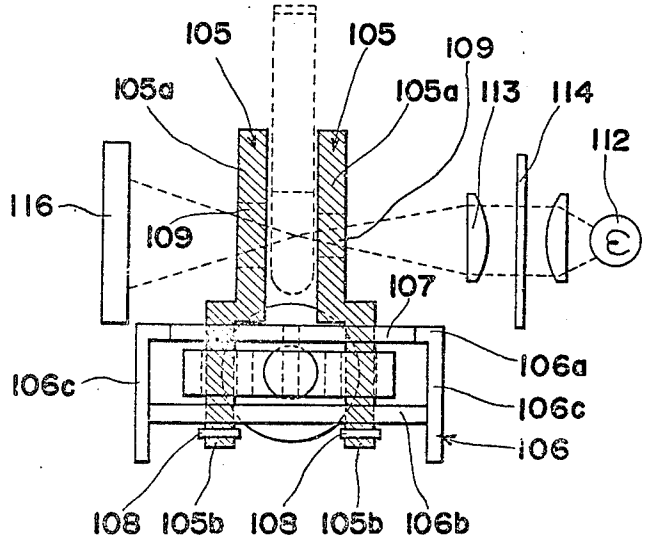


FIG. 12

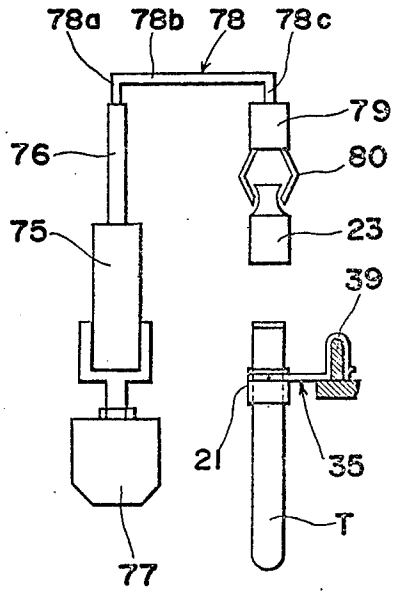
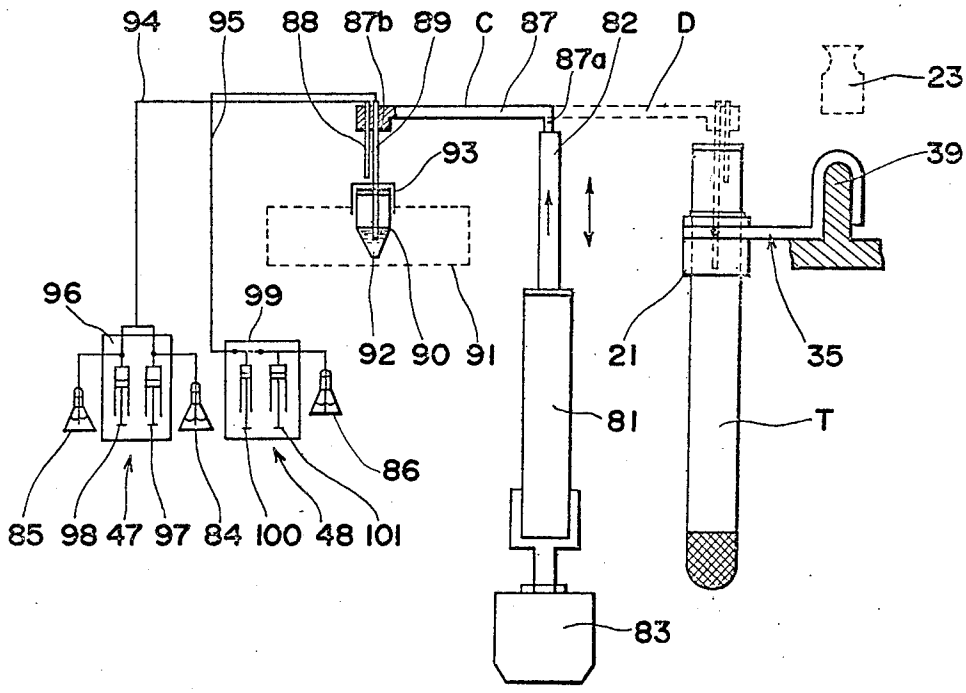


FIG. 13


94
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101
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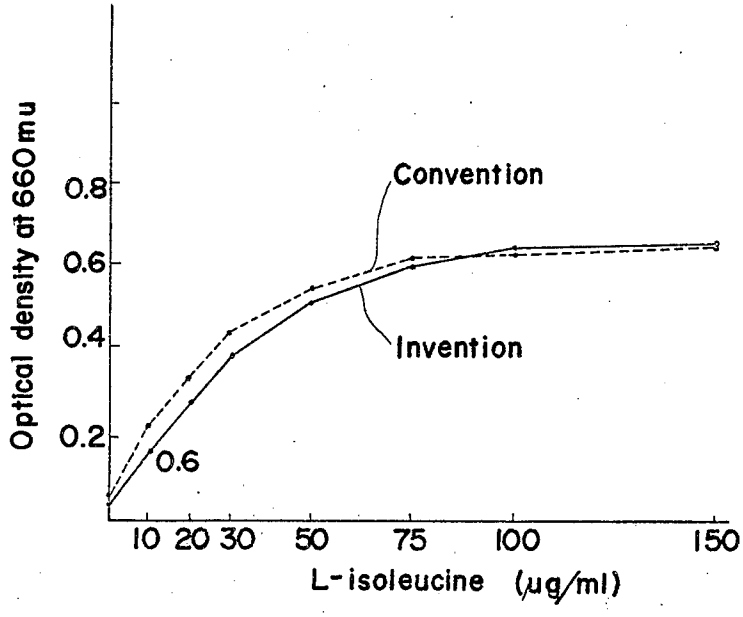
FIG. 16


Optical density at 660mu
0.8
0.6
0.4
0.2
0.6
Convention
Invention
10
20
30
50
75
100
150
L-isoleucine (μg/ml)

METHOD OF CONTINUOUS AUTOMATIC INCUBATION OF BACTERIA AND DETERMINATION OF GROWTH THEREOF, AND APPARATUS THEREFOR

The present invention relates to a method for culturing bacteria with shaking and to an apparatus employed in said method, and more particularly to an improved method and means for providing a good air supply to aerobic microorganisms to accelerate the growth thereof.

Microorganisms are frequently employed in the development or production of medical or chemical compounds, or for microbioassay of amino accids and vitamins as well as for test for activities of antibiotics. In order to promote the growth of aerobic microorganisms it has been known to incubate the microorganisms in a medium with a constant supply of oxygen, which is commonly effected by shaking the culture and the medium. In conventional shaking methods and means, cultures may be placed in test-tubes which are then shaken or rotated. Conventional methods and means have the disadvantage that if the test-tubes are held vertically, oxygen supply to the cultures is poor, since, in a test-tube, the surface area exposed to the air is small, and even if the test-tubes are inclined in order to increase this surface area, the amount of oxygen moving into the cultures still remains small. When attempts are made to overcome this problem by vigorous shaking of the test-tubes, there is frequently leakage and loss of culture broth past stoppers.

In addition, processes involved in the microbiological growth experiments are injection of a culture medium and sample solutions, inoculation, incubation, and determination of the bacterial growth. Because of the complexity of the above-described processes, it is highly desirable that the processes be automated as fully as possible. However, there is no overall system with a degree of automation permitting research to advance smoothly and with minimum of attention by staff. In conventional methods and means two types of apparatus are available: the one only provides for automatic incubation of cultures and determination of the growth at certain intervals; and the other is for automatic injection of sample solution and medium and determination of the growth at a certain time thereafter. The former type suffers from the disadvantages that the sample handling capacity is so limited that it is not possible to simultaneously test a large number of samples.

It is accordingly an object of the present invention to provide a method and an apparatus that overcome the defects inherent in conventional methods and means for shaking culture of microorganisms.

It is another object of the present invention to provide a method and an apparatus that makes a considerable contribution to facilitating and accelerating bacterial growth without loss or leakage of the culture. In addition to increased productivity, the method and apparatus of the invention offer the advantages of easy adaptation to use for different cultures, and rapid adjustability of degrees of shaking. Further, the apparatus of the invention is simple and low-cost.

According to the invention there is provided a frame on which a plurality of holders, wherein test-tubes containing cultures may be held, are rotatably mounted. The frame is mounted on horizontal, parallel bars, and

is freely adjustable forwards or rearwards thereon. The horizontal bars are mounted on vertical support columns, and are freely adjustable upwards or downwards thereon. A horizontal bar is caused to move reciprocally by drive means, and to strike the suspended test-tubes at certain points thereon, whereby the test-tubes are caused to shake, and the cultures therein are shaken. The invention offers the advantages that the effective stroke length of the bar causing test-tubes to shake can be easily and rapidly adjusted, that the shaking frequency of the test-tubes is easily adjusted be adjusting the speed of the bar drive means, and that therefore the exact degree of shaking of the cultures is adjustable and controllable. In addition, the method and apparatus of the invention can provide more vigorous shaking than that achieved in conventional methods and means.

It is a further object of the present invention to provide an automated method and an apparatus for injection of medium, inoculation, incubation with or without shaking, and repeated determination of the growth of bacteria, all in a continuous process, whereby the bacterial growth can be observed in detail and more accurately.

It is a still further object of the present invention to provide a method and an apparatus which makes possible a continuous process wherein sample solutions are diluted, and culture medium and said sample solutions are supplied to test-tubes in an aseptic, constant-temperature chamber, the mixture of sample solution and culture medium is automatically inoculated, said test-tubes are transported on a zig-zag rail and shaken by a reciprocating bar while being so transported, and the bacterial growth is determined by a turbidimetric method at repeated intervals directly in said test-tubes.

It is a still further object of the invention to provide an apparatus wherein a zig-zag guide rail, the length of which is adjustable, is provided in an aseptic, constant-temperature chamber, test-tubes are freely suspended in holders pivotally mounted on said guide rail, a means for causing said holders to move on said guide rail are provided at certain points adjacent said guide rail, a means for injection of sample solutions and culture medium into said freely suspended test-tubes is provided at a station adjacent to said guide rail, said test-tubes are shaken by a bar driven by a drive means while being transported on said guide rail, an assessment station, wherein said test-tubes may be halted and the optical density of the cultures therein may be measured in order to determine the bacterial growth, is provided by said guide-rail, whereby test-tubes containing cultures may be transported over a path adjustable length while being shaken and the growth may be determined directly from said test-tubes at variable intervals in a continuous, automatic process.

These and other objects and features of the present invention will become apparent from the following description taken in conjunction with preferred embodiments thereof with reference to the accompanying drawings in which:

FIG. 1 is a plan view of an apparatus for the shaking of cultures in accordance with one preferred embodiment of the present invention,

FIG. 2 is a side elevational view of the apparatus of FIG. 1,

FIG. 3 is a perspective view of a support frame for a test tube employed in the apparatus of FIG. 1,

FIG. 4 is a perspective view of a holder for a test tube similar to that of FIG. 3, according to another embodiment of the present invention,

FIG. 5 is a front view of a shaking test tube, for purpose of illustrating an experimental method according to the present invention,

FIG. 6 is a graph showing results of an experiment employing the apparatus of FIG. 1 and applying the method of the present invention,

FIG. 7 is a schematic plan view showing a layout of apparatuses for incubation in accordance with another preferred embodiment of the present invention,

FIG. 8 is a cross-sectional view of a drive means **42** employed in the layout of FIG. 7,

FIG. 9 is a plan view of the drive means of FIG. 8,

FIG. 10 is a side-elevational view of a fast advance means **43** employed in the layout of FIG. 7,

FIG. 11 is a plan view of a bar drive means **52** employed in the layout of FIG. 7,

FIG. 12 is a side-elevational view of a means **46** for removing and replacing caps employed in the layout of FIG. 7,

FIG. 13 is a schematic side-elevational view of a supply means employed in the layout of FIG. 7,

FIG. 14 is a plan view of a test-tube holding means **49** employed in the layout of FIG. 7,

FIG. 15 is a cross-sectional view of the holding means of FIG. 14, and

FIG. 16 is a graph showing results of an experiment employing the apparatus of FIG. 7, and applying the method of the present invention.

Before the description proceeds, it is to be noted that like parts are designated by like reference numerals throughout the accompanying drawings for the sake of brevity. It is to be noted that the concept of the present invention as well as its application to equipment for the continuous shaking of a test-tube herein disclosed as a preferred embodiment can be applied in any field of industry other than the medical industry, for example, in connection with liquid, oil or other like fluid if an amount of such fluid is inserted into a bottle or other like receptacles.

Referring first to FIGS. 1 and 2, the apparatus for shaking a culture of micro-organisms to which the present invention is applicable comprises a support base **10**, two support columns **11** fixedly implanted in the base **10** near opposite sides thereof, and extending vertically upwards therefrom, and a flat bar **12** is mounted on each support column **11**, at right-angles thereto, that is, parallel to the support base **10**. The flat bars **12** are also mounted parallel to one another, and extend in the direction of the front of the support base **10**. Near one end of each bar **12**, there is formed a hole **13**, which possesses a diameter slightly larger than that of a support column **11**, and permits the bar **12** to be slidably mounted on the corresponding support column **11**. In other words, the bars **12** can be moved vertically upwards or downwards to any desired position on the columns **11**. Each bar **12** is held in a desired position on the corresponding column **11** by a bolt **14**, which screws into a threaded hole **15** formed in the bar end, and contacts the side of the column **11**.

A test-tube support frame **16** is mounted on the flat bars **12**. The support frame **16** comprises a long main bar **16***a*, and a plurality of short bars **16***b* which are formed integrally with the main bar **16***a*, and extend at right-angles thereto. The length of the support frame main bar **16***a* is approximately equal to the distance between the support columns **11**, and each end of the bar **16***a* has therein a slot **17**, into which the flat bar **12** fits slidably, as shown in FIG. 3. In other words, the support frame **16** can be moved horizontally forwards or rearwards along the flat bars **12** to any desired position. The frame **3** is fixed in a desired position by means of bolts **18** each of which fits into a threaded-opening **19** formed in the support frame end, and contacts the side of the corresponding bar **12**.

A pin **20** is fixedly attached to and projects from each side of each short bar **16***b*, near the outer end thereof, that is, the end further removed from the main bar **16***a*. A hollow, cylindrical holder **21** is provided between each pair of short bars **16***b*, each holder **21** being mounted on a pair of pins **20** and being free to rotate thereon. A test-tube T provided with a rubber ring **22** is held in each holder **21**, the test-tube T being passed through the ring **22** and holder **21**, and the test-tube T being supported by the rubber ring **22** in holder **21**. The ring **22** being adjustable upwards or downwards, the seating of the test-tube T can be made higher or lower in the holder **21**. Also, since each holder **21** is rotatably mounted on pins **20**, each test-tube T can be swung forwards or rearwards relative to the main bar **16***a*. Since there is plurality of short bars **16***b*, and holders **21**, a plurality of test-tubes T may be rotatably supported by the support frame **16**. Each test-tube T is covered by a cap **23**.

A motor **24** is provided on the support base **10**. The motor **24** is directly connected to a first wheel **25**, which is positioned above the motor **24**, and lies parallel to the surface of the base **10**. A second wheel **26** level with the first wheel **25** is rotatably mounted on a shaft projecting upwards from the base **10**. Power is supplied to the second wheel **26** from the motor **24**, for example by a chain sprocket **27**, and a chain **28** which transmits drive to another chain sprocket **29** mounted coaxially with the second wheel **26**. When, therefore, the motor **24** is actuated, both wheels **25** and **26** are rotated synchronously at the same speed. The description continues below with reference to the first wheel **25** and elements associated therewith, it being understood that the description also applies to the second wheel **26**. In the upper surface of the wheel **25**, there is formed a radial groove **25***a*, which extends from the center to the periphery of the wheel **25**, and accommodates one end of a crankshaft **30**. The crankshaft end is slidably mounted in the groove **25***a*, and is fixed at a desired point therein by a holding pin **31**. The stroke of the crankshaft **30** during rotation of the wheel **25** may therefore be varied by adjustment of the position of the crankshaft end in the groove **25***a*, the stroke being longer the nearer the crankshaft end is to the periphery of the wheel **25**. The other end of the crankshaft **30** is rotatably connected to one end of a piston **32** by a connecting pin **117**. The piston **32** extends forwards, below the support frame main bar **16***a*, and is slidably supported by a bearing block **33**, which is mounted on the base **10**. The piston **32** is caused to slide through the bearing block **33**, and is driven in a reciprocating motion together with the crankshaft **30** by rotation of the wheel **25**. The other, forward end of the piston has fixedly attached thereto a long bar **34**, near one end thereof. The forward end of a second piston **32**, which is connected through a second crankshaft **30** and holding pin **31** to the second wheel **26**, is fixedly attached to the bar **34**, near the other end thereof. The bar **34** is approximately the same length as the support frame

main bar **16***a*, and contacts the lower portions, that is, below the holders **21**, of test-tubes T held in the frame **16**. When, therefore, the motor **24** is actuated, the wheels **25** and **26** are rotated, the crankshafts **30** and pistons **32** are driven in a reciprocating motion, the bar **34** is also moved successively forwards and rearwards, and the test-tubes T are rocked by the repeated pushes of the bar **34**. This rocking of the test-tubes T effectively shakes the contents thereof.

There are no special restrictions concerning the material of the bar **34**. The bar **34** is preferably made of a material with a high rigidity, which may be, for example, steel or iron. In consideration of possible damage to test-tubes T, the bar **34** is preferably encased in a tube, of vinyl or similar material. However, such a covering should not be thick, as this lowers the effectiveness of the rocking action by the bar **34**.

It is to be noted that, according to the present invention, it is not essential that there be provided a unitary frame for holding test-tubes T, but there may be provided detachable supports **35**, as shown in FIG. **4**. Each test-tube support **35** comprises a yoke **36**, to one side of which a U-or V-shaped forked portion **37** is fixedly attached. The yoke **36** and forked portion **37** may of course be formed integrally. Pins **20** are fixedly attached to the arms of the forked portion **37**, near the outer ends thereof. The pins **20** support and permit rotation of a test-tube holder **21**. The yoke **36** is hooked onto a rail **39**, whereby the holder **21** is mounted in a desired position, and a test-tube T in the holder **21** may be rocked in the same manner as described above. Since each support **35** is a separate unit, yokes **36** may be slid along the rail **39**, and test-tubes T may be rocked while being simultaneously transported to some required stage. Also, it is possible to mount a plurality of supports **35** on parallel rails, and test-tubes T supported by the supports **35** on different rails may be simultaneously rocked by connected bars **34**.

The means of the invention offers the particular advantage that adjustment of the degree of shaking of the contents of test-tubes T is easily and rapidly effected. The degree of shaking of the contents of test-tubes T depends on the amount of the frequency of rocking of the test-tubes T, and this may be varied by varying the speed of the motor **24**, and by adjusting the positions of the crankshaft ends to vary the length of the stroke of the bar **34**. Adjustment of the effective stroke length of the bar **34** may also be made by moving the support frame **16** forwards or rearwards on the flat bars **12**, or by moving the flat bars **12**, and therefore the support frame **16**, upwards or downwards on the support columns **11**. For example, to obtain the maximum shaking effect, the crankshaft ends are fixed by the holding pins **31** at the outer peripheries of the wheels **25** and **26**, the position of the support frame **16** is adjusted so that test-tubes T therein are pushed at the center point of the reciprocal stroke of the bar **34**, and the rotational speed of the motor **24** is adjusted, to make the period of the reciprocating motion of the bar **34** double the oscillatory period of the test-tubes T. It is to be noted, however, that, in cases of extreme shaking, if test-tubes T are swung by more than 50°, there is a risk of the contents being thrown out therefrom. Therefore, there may be provided a stop **38** fixed on the support base **10** on the opposite side of the test-tubes T from the bar **34**, in order to prevent test-tubes T from being swung more than 50°. It is, of course, possible to set up a plurality of frames **16** parallel to one another, and to simultaneously rock test-tubes T in the frames **16** by interconnected bars **34**.

Experiments, which are described below, were carried out to compare the effectiveness of the means of the invention with that of conventional shaking culture means.

EXPERIMENT I

There were employed uniform test-tubes T having dimensions of 15 × 150 mm and a weight of 15.5 g. In each test-tube T there was placed a culture medium whose composition was comprised of 20 % sorbitol, 1 % corn steep liquor and 0.03 % calcium carbonate. The medium was inoculated with *Acetobacter suboxidans*, and the test-tubes were covered by caps having dimensions of 20 × 47 mm and a weight of 6.5 g. The test-tubes T were supported at 35 mm from their upper ends by O rings attached therearound, and were suspended in holders **21**. The test-tubes T were struck cyclically at a point 35 mm from the bottom by an iron bar which was 15 mm in diameter, was covered by a covering of rubber 1 mm thick, and had a stroke length of 40 mm. The impact frequency was varied, that is, the number of cycles per minute of reciprocal motion of the bar was varied, and the rate of sorbose production from sorbitol for 24 hours at 30°C, was measured. At the same time the angular displacement θ, as shown in FIG. **5** of the test-tubes T at the different impact frequencies were measured. For the purposes of comparison, cultures comprised of the same constituents were shaken by a conventional, commercially available reciprocal shaker, having a stroke of 85 mm and an impact frequency of 149 cpm, for 24 hours at 30°C.

The results of the experiment, which are plotted in FIG. **6**, showed that sorbose production was accelerated with increased impact frequency up to a peak of about 150 cpm, over which there is a decline. The ratio of sorbose produced to sorbitol added at 150 cpm was 75%, which is an outstandingly marked improvement as compared with a ratio of 40% attainable with the conventional means. It was therefore made clear that far superior results are obtained by the shaking means of the invention.

It was also established that the rate of production of sorbose is in a positive relationship to the angular displacement of the test-tubes T, that is, production increases as the angle of displacement θ increases. At an impact frequency of 150 cpm, the frequency of reciprocal motion of the bar **34** is exactly twice that of the shaking motion of the test-tubes T, and the bar **34** strikes the test-tubes T at the center of their reciprocal motion (θ=0°). At this point the momentum of both bar **34** and test-tubes T is maximum. The increased rate of production of sorbose under these conditions may be considered to be due to the fact that the impact imparted to cultures within the test-tubes T is maximum, displacement of test-tubes T is large, and an increased amount of oxygen is transferred into the cultures. The lower rates of sorbose production at impact frequencies above or below 150 cpm are probably due to the fact that the impact imparted to the test-tubes is less since the bar does not strike the test-tubes T at the center of its reciprocal motion.

EXPERIMENT II

The conditions of this experiment were the same as those of Experiment I, except that bars of different materials were used. Results

No.	Bar Material	Results Sorbose Production (%)
1	Bare iron	80
2	Iron covered with rubber 1 mm thick (Experiment I)	75
3	Iron covered with rubber 3 mm thick	60

As is clear from the above description, the present invention provides a shaking method and means whereby bacterial growth is remarkably increased, without loss or leakage of said cultures.

Referring now to FIGS. 7 to **15,** the incubation and determination means according to another embodiment of the invention may be seen to comprise a guide rail **39** housed in a chamber **40,** wherein there is maintained as aseptic atmosphere at a constant temperature by a means **41** provided adjacent thereto. The guide rail **39** is a continuous rail having a straight section **39-1** and a series of loops **39***a*, **39***b*, **39***c*, which are generally at right-angles to the straight section **39-1** and each of which comprises two comparatively long, straight, parallel sides.

In this embodiment of the invention, the guide rail **39** has three (first, second and third) loops **39***a*, **39***b*, **39***c*. Needless to say, however, the number of loops may be any number required. At the top end of each loop **39***a*, **39***b* and **39***c*, that is, the end thereof further removed from the straight section **39-3, 39-5** . . . **39-13,** there is provided a drive means **42** on the inside of the curve. The straight section **39-1** joins the bottom end of the outer side of the first loop **39***a* to the bottom end of the outer side of the third loop **39***c*. A drive means **42** is provided on the inside of the curve where the staight section **39-1** meets the first loop **39***a*, where the straight section **39-1** meets the third loop **39***c*, and also in approximately the center of the straight section **39-1,** that is, generally opposite the bottom end of the second loop **39***b*. Between the first and second loops **39***a* and **39***b*, near the lower ends thereof and on the inside of the curve formed by a guide rail **39-6,** which joins the bottom of the inner side of the first loop **39***a* to the bottom of one side of the second loop **39***b*, there is provided a fast advance means **43.** Another fast advance means **43** is similarly provided between the second and third loops **39** *b* and **39***c*, on the inside of the curve formed by a guide rail **39-10** joining the bottom of the outer side of the second loop **39***b* to the bottom of the inner side of the third loop **39***c*. Test-tubes T held in holders **35** of the same construction as that shown in FIG. **4** of the first embodiment, the yoke **36** of which is slidable on the guide rail **39,** are transported, in a manner described below, along the straight section **39-1,** up the outer side **39-3** of the first loop **39***a*, down the inner side **39-5** of the first loop **39***a*, around the second loop **39***b*, up the inner side **39-11** of the third loop **39***c* and down the outer side **39-13** of the third loop **39***c*, and back to the straight section **39-1.** In other words the test-tubes T follow a generally zig-zag path. The length of the path over which the test-tubes T are carried may be shortened by providing a guide rail shunt section **44** as shown with a dotted line in FIG. **7** to join the straight section **39-1** directly to the bottom of the second loop **39***b*, on the side thereof lying nearer the first loop **39***a*. In this case, the test-tubes T are carried half-way along the straight section **39-1** up the shunt section **44,** around the second loop **39***b*, around the third loop **39***c*, and back to the straight section **39-1.** The length of the path over which test-tubes T are transported may be still further shortened by joining the straight section **39-1** directly to the bottom of the inner side of the third loop **39***c* by a shunt section **45** as shown with a dotted line in FIG. **7.** In this case, test-tubes T simply travel around the third loop **39***c*. Whatever the length of the path over which test-tubes T are transported, the test-tubes T are initially brought to a supply station I, and finally brought to an assessment station II. The supply station I is situated generally opposite the bottom end of the third loop **39***c*, and comprises a means **46** for removing and replacing caps, a means **47** and **48** for supplying culture medium, sample solutions, inoculum and any other required substances, into test-tubes T. The assessment station II is situated opposite the bottom of the outer side of the third loop **39***c*, and comprises a test-tube holding means **49,** an electrophotometer **50,** and a recording unit **51.** Parallel to the straight side portions of each loop **39***a*, **39***b* and **39***c* there are provided reciprocating long bars **34***a*. That is, there is one pair of bars **34***a* in association with each loop **39***a*, **39***b* and **39***c*, the bars **34***a* in each pair being on opposite sides of the corresponding loop **39***a*, **39***b* and **39** *c*. The bar **34***a* facing the outer side of the third loop **39***c* is divided into two independent sections **34***b* and **34***c*, the bar section **34***b* being longer and facing the upper portion of the outer side of the third loop **39***c*, and the bar section **34***c* being shorter and facing a lower portion thereof. A single reciprocating long bar **34***d* is provided parallel to the guide rail straight section **39-1.** Each pair of bars **34***a* and **34***b* and **34***c*, is indirectly supported by, and driven by a drive means **52,** which is located inside the corresponding loop **39***a* and **39***b* and the single bar **34***d* is driven through a linkage **53** thereto. The short bar **34***c* is driven by an independent drive means **74.**

The above-mentioned holders **35,** drive assemblies **52,** bars **34,** drive means **42,** and fast advance means **43** will be described in further detail hereinbelow. Unless otherwise specified, the description will be in reference to single means **35, 42, 43,** or set of means **52, 34,** it being understood that there is a plurality of indentical means **35, 42, 43,** or set of means **52, 34.**

Referring now to FIGS. **4** and **8,** the holder **35** may be seen to comprise a yoke **36,** and a forked section **37,** which is integral with and generally at right-angles to one side of the yoke **36.** On the other side of the yoke **36** there is fixedly attached a projecting stud **54,** which is engaged by drive means **42,** or fast advance means **43,** as described below. The yoke **36** is hooked onto the guide rail **39,** whereby the holder **35** is slidably supported thereby. In this configuration, the forked section **37** is generally horizontal. A pin **20** is provided at the end of each arm of the forked section **37.** A hollow cylindrical support **21** is rotatably mounted on the pins **20,** and between the arms of the forked section **37.** On the upper portion of the test tube T there is provided a rubber ring **22,** the setting of which relative to the support **21** is adjustable upwards or downwards, and which acts as a support pad for the upper portion of the cylindrical support **20.** A test-tube T to be transported around the guide rail **39** is slid downwards through the ring **22** and hollow cylindrical support **21,** and is held suspended thereby. The amount by which the rubber ring **22** stands clear of the cylindrical support **21** being adjustable, the setting of the test-tube T may be ad-

justed upwards or downwards relative to the holder **35**, and hence relative to the guide rail **39**. When the holder **35** is in repose on the guide rail **39**, the test-tube T is held suspended in a vertical position. However, since the cylindrical support **21** may pivot on the pins **20**, and swing freely in the arms of the forked section **37**, the test-tube T also may be swung freely, towards or away from the guide rail **39**.

In FIG. **7** there are shown only a few test-tubes T suspended in holders **35** hooked on the guide rail **39**. In actual use of the automated culture means of the invention there are test-tubes T in holders **35** over the whole length of the guide rail **39**, except for the portions **39-6** and **39-10** thereof facing the fast advance means **43**. When being transported around the guide rail **39**, test-tubes T contain bacterial cultures supplied thereinto at the supply station I. Since it may not be possible to maintain a completely aseptic atmosphere in the chamber **40**, and there may be remnant bacteria, even in asceptic air supplied by the means **41**, and since such remnant bacteria could contaminate cultures contained in the test-tubes T, especially if the cultures are of low activity, each test-tube T is covered by a cap **23**, to further protect the contents thereof.

Referring to FIG. **8**, the drive means **42** comprises a horizontal wheel **55**, which rotates about a vertical axis **56**, and which is driven by a rotary solenoid **57**. Indentations **55***a* are formed at even intervals in the outer periphery of the wheel **55***a*. The indentations **55** engage studs **54** projecting from the sides of yokes **36** of the holders **35**, whereby rotation of the wheel **55** may cause holders **35** to be driven around curved portions of the guide rail **39**. For example, referring more particularly to FIG. **7**, the wheel **55** at the top of the first loop **39***a* follows the curve of the guide rail **39-4** over a semicircular arc. When, therefore, a holder **35** carrying a test-tube T comes to the top of the outer side of the first loop **39***a*, the stud **54** on the yoke **36** thereof is engaged by an indentation **55***a* in the wheel **55** at the top of the first loop **39***a*, and the rotary solenoid **57** being actuated and the wheel **55** rotated, the holder **35** is carried 180° around the top end of the first loop **39***a*, and to the top of the inner side thereof. When the holder **35** is thus carried around the top of the first loop **39***a* it pushes the preceding holder **35** forwards, and is itself subsequently pushed forward by the succeeding holder **35** carried around the top of the first loop **39***a* by engagement of the stud **54** thereof by the succeeding indentation **55***a* of the wheel **55**. Holders **35** are similarly carried through 180° around the tops of the second and third loops **39***b*, **39***c* successive holders **35** pushing preceding holders **35** forwards. The wheels **55** of the drive means **42** provided at the curved sections where the guide rail straight section **39-1** meets the first and third loops **39***a* and **39***c* follow the curve of the guide rail **39-2** and **39-14** through 90° only. At these points thereof, holders **35** are moved forwards in the same manner as described above. If, however, a shunt section **45** is installed between the straight section **39-1** and the third loop **39***c*, the wheel **55** at that location carries holders **35** through 180°, from the bottom of the outer side of the third loop **39***c* to the bottom of the inner side thereof. The wheel **55** of the drive means **42** provided by the guide rail straight section **39-1**, at the bottom of the second loop **39***b*, normally advances holders **35** in a straight line only, along the straight section **39-1**. If, however, a shunt section **44** is installed between the straight section **39-1** and the second loop **39***b*, the wheel **55** at that location carries holders **35** though 90°, from the straight section **39-1**, onto the shunt section **44**, successive holders **35** pushing preceding holders **35** upwards on the second loop **39***b*. All the drive means **42** are actuated synchronously, whereby test-tubes T are transferred along the guide rail **39** at the same speed at each location of a drive means **42**.

As shown in FIGS. **9** and **10** the fast advance means **43** comprises a cylinder **58** and plunger **59** assembly, and a rotary solenoid **60**. The cylinder **58** and plunger **59** assembly is positioned vertically, and is connected to, and rotated in a horizontal plane by the rotary solenoid **60**, which is provided therebelow. The outer end of the plunger **59** is fixedly connected to the end of one section of a bracket-like arm **61**. The arm **61** comprises a vertical section **61***a*, connected to the plunger **59**, a horizontal section **61***b*, and a vertical section **61***c*. The section **61***c* extends vertically downwards, and on the inside of the bottom end thereof there is formed a groove **62**. The groove **62** is for engagement of studs **54** on holders **35**. The arm **61** being connected to the plunger **59**, when the cylinder **58** and plunger **59** assembly is actuated to raise or lower the plunger **59**, the arm **61** is likewise raised or lowered. Also, when the rotary solenoid **60** rotates the cylinder **58** and plunger **59** assembly, the arm **61** is likewise rotated in a horizontal plane.

In the starting position of, for example the, fast advance means **43** between the first and second guide rail loops **39***a* and **39***b*, the plunger **59** and arm **61** are raised, and the arm section **61***c* lies over point A, that is, the start of the curved section **39-6** between the first and second loops **39***a* and **39***b*. When a holder **35** pushed by other holders **35** comes to point A, the cylinder **58** and plunger **59** assembly is actuated to retract the plunger **59** and lower the arm **61**, and the groove **62** of the section **61***c* slides onto, and engages the stud **54** of the holder **35**. Next, the rotary solenoid **60** is actuated to swing the cylinder **58** and plunger **59**, and arm **61** through 180°, so that the arm end section **61***c* goes from point A to point B. Engagement of the groove **62** with the holder stud **54** causes the holder **35**, and test-tube T held thereby, to be similarly swung along the curve A-B. When the holder **35** is brought to point B it pushes the preceding holder **35** forward. The cylinder **58** and plunger **59** assembly is now actuated to push the plunger **59** upwards, whereby the arm **61** is raised, and the groove **62** disengages from the stud **54**. Next, the solenoid **60** swings the arm **61** back through 180°, to bring the section **61***c* back to point A, ready to engage the next holder **35**, which has been pushed forward to point A. The above-described operation is then repeated, and this next holder **35** is transferred rapidly to point B. Holders **35** are transferred in the same manner and at the same speed over the curved section A-B of the curved section **39-10** between the second and third loops **39***b*, **39***c*.

The operation of the drive means **42** and fast advance means **43** are adjusted and synchronized so that the time required for transfer of holders **35** over all curved sections **39-2, 39-4, . . . 39-14**, of the guide rail **39** is the same, whereby a smooth flow is maintained. The rate of advance of test-tubes T is also such that test-tubes T may be halted for 30 second intervals, to permit injection of sample solution and inoculation thereinto at the supply station I, and determination of the growth of bacteria at the assessment station II.

Referring to FIGS. 8 and 11, the drive means 52 for actuating long bars 34 may be seen to comprise connecting rods 63, crank-shafts 64, and horizontal wheels 65. The connecting rods 63 are horizontally and slidably supported in slide bearing blocks 66, which are fixedly mounted on the base of the chamber 40. Opposite ends of the rods 63 are fixedly attached to bars 34*a* on opposite sides of a guide rail loop 39, the connecting rods 63 being generally at right-angles to the bars 34*a*. The connecting rods 63 slide in the bearing blocks 66 and permit the bars 34*a* to be moved towards or away from the guide rail 39. Also, because of the connecting rods 63, movement of one bar 34*a* causes the other bar 34*a* to move in the same manner. The ends 64*a* of two crank-shafts 64 are rotatably attached to one bar 34*a*. The other ends 64*b* of the crank-shafts 64 are rotatably attached to wheels 65 by holding pins 67. Rotation of the wheels 65 therefore causes the crank-shafts 64, and bar 34*a* attached thereto, to move in a reciprocating motion. Each pin 67 is adjustably fixable in a radial groove 68 formed in the upper surface of a wheel 65. In other words, the end 64*a* of each crankshaft 64 may be moved to, and fixed at different locations between the center and the periphery of the corresponding wheel 65, whereby the length of the stroke of the crankshaft 64, and hence bar 34*a*, may be varied. One wheel 65 is fixedly mounted on and driven by the drive shaft 69 of a motor 70. The other wheel 65 is fixedly mounted on a rotatable shaft 71, which is mounted vertically on the base of the chamber 40, and on which a chain sprocket 72 is mounted. A chain sprocket 72 is also mounted on the motor drive shaft 69. The chain sprockets 72 engage a chain 73 connecting the drive shaft 69 and shaft 71. Therefore, when the drive shaft 69 is rotated, the crank-shafts 64, and the bar 34*a* attached thereto are driven reciprocally, the bar 34*a* being moved towards or away from the guide rail 39. When the bar 34*a* is thus driven reciprocally, it cyclically strikes the lower portions of test-tubes T suspended in holders 35 on one side of the loop 39, whereby the test-tubes T are caused to shake, and the cultures therein are shaken, and the supply of oxygen thereto is increased. The degree of shaking of cultures in test-tubes T may be varied by adjustment of the stroke length of the bar 34*a*, that is, by fixing crank-shaft ends 64*b* nearer or further away from the peripheries of wheels 65, to lengthen or shorten the stroke of the crank-shafts 64. The bar 34*a* attached to the crankshaft ends 64*a* being driven reciprocally, the bar 34*a* on the other side of the loop 39 is similarly driven, because of the connection of the rods 63 sliding in the bearings 66, and this bar 34*a* also strikes test-tubes T in the same manner, and producing the same results.

Other bars 34 associated with other guide rail loops 39, including the bar sections 34*b* of the third loop 39*c*, or the bar 34*d* opposite the guide rail straight section 39-1, are similarly supported, connected and driven by connecting rods 63 sliding in bearing blocks 66 and crank-shafts 64 driven reciprocally by wheels 65. Chain sprockets 72 are mounted on the rotary shafts 71 of all wheels 65, and are engaged, and connected by chains 73. All wheels 65 are therefore driven synchronously by the motor 70, whereby all bars 34 are driven in synchronous reciprocal motion so that all test-tubes T on the guide rail 39 are caused to shake with the same frequency. The short bar section 34*c* on the path of test-tubes T immediately before the assessment station II is further provided with an independent drive means 74, whereby the bar section 34*c* may be driven in reciprocal motion even when the bar section 34*b* and other bars 34*a* are undriven and remain stationary.

It is to be noted that various modes of operation are possible with the apparatus of the invention. Test-tubes T containing cultures may be transported around the guide rail 39 by the action of the drive means 42 and fast advance means 43, and simultaneously caused to shake by the bars 34. Alternatively, the action of the drive means 42 and fast advance means 43 may be stopped and only the bars 34 be actuated, whereby test tubes T are shaken, while test-tubes T remain in the same locations. Also if the short bar section 34*c* only is actuated, test-tubes T may be transported around most of the guide rail 39 without being shaken, and then be agitated for a short time only immediately prior to coming to the assessment station II, whereby bacterial cells in the test-tubes T are evenly mixed.

Referring to FIG. 12, the means 46 for removing and replacing caps 23 comprises an electrically-driven cylinder 75 and plunger 76 assembly, and a rotary solenoid 77. The cylinder 75 and plunger 76 assembly is positioned vertically, and is connected to, and rotated in a horizontal plane by the rotary solenoid 77, which is provided therebelow. The outer end of the plunger 76 is fixedly connected to the end of one section of a bracket-like arm 78. The arm 78 comprises a vertical section 78*a*, connected to the plunger 76, a horizontal section 78*b*, and vertical section 78*c*. The vertical section 78*c* extends vertically downwards, and at the outer end thereof thereof there is provided an electromagnet 79 and a grab 80, which is controlled by the electromagnet 79. When a test-tube T in a holder 35 is brought to the position of the cap 23 removal and replacement means 46, it comes under the grab 80. The cylinder 75 and plunger 76 assembly is then actuated to draw the plunger 76 downwards and into the cylinder 75, thus lowering the arm 78 and grab 80. When the grab 80 has been lowered to a position around the cap 23 on the test-tube T, the electromagnet 79 is actuated to close the grab 80, which therefore grips the cap 23. While the grab 80 continues to grip the cap 23, the cylinder 75 and plunger 76 assembly is actuated to raise the plunger 76 and arm 78, whereupon the cap 23 is removed from the test-tube T by the grab 80. The rotary solenoid 77 is now actuated to rotate the cylinder 75 and plunger 76 assembly and arm 78, and swing the cap 23 out of alignment with the test-tube T. Culture medium, sample solution and inoculum are now supplied into the test-tube T by the supply means 47 and 48, as described below. When this supplying process is completed, the rotary solenoid 77 is again actuated to rotate the cylinder 75 and plunger 76 assembly and arm 78, and swing the cap 23 back into alignment with the test-tube T. Next the cylinder 75 assembly is actuated to move the plunger 76 and arm 78 downwards, and bring the cap 23 back onto the test-tube T. After this, the electromagnet 79 opens the grab 80, which therefore releases the cap 23, and then, the plunger 76, arm 78, and grab 80 are raised vertically, in readiness for the next test-tube T, and the cap 23 is left on the first test-tube T, to protect the culture therein.

When a cap 23 is removed from a test-tube T, as described above, requisite materials are supplied into the test-tube T by the above-mentioned supply means 47 and 48, which are shown in FIG. 13. The supply means 47 and 48 comprise an electrically driven cylinder 81 and plunger 82 assembly, and a rotary solenoid

83. There are also separately prepared supplies of culture medium 84, inoculum 85, and water 86. The cylinder 81 and plunger 82 assembly is positioned vertically, and is connected to, and rotated in a horizontal plane by the rotary solenoid 83, which is provided therebelow. One end 87*a* of an arm 87 is fixedly attached to the outer end of the plunger 82. The arm 87 is aligned horizontally, and the other end 87*b* thereof supports nozzles 88 and 89, which are directed vertically downwards, the nozzle 88 being shorter than the nozzle 89. When the rotary solenoid 83 rotates the cylinder 81 and plunger 82 assembly, the arm 87 is likewise swung in a horizontal plane. The arm 87 is swung so that the outer end 87*b* thereof, and nozzles 88 and 89, supported thereby, come to one of two positions C and D. In position C, as shown with solid lines in FIG. 13, the arm end 87*b* and nozzles 88 and 89 are vertically above a sample solution container 90 in a sampling unit 91. The sampling unit 91 contains a plurality of containers 90, which are moved successively into alignment with nozzles 88 and 89 in position C. Each container 90 holds sample solution 92, and is covered with a sheet of aluminum foil 93. In position D, as shown with dotted lines in FIG. 13, the arm end 87*b* and nozzles 88 and 89 are vertically above a test-tube T, from which the cap 23 has been removed.

The nozzles 88 and 89 are connected by flexible supply lines 94 and 95 to the supply means 47 and 48, respectively. The supply means 47 includes an injector 96 comprising two microcylinders 97 and 98, which are connected to branch lines of the supply line 94. The microcylinder 97 is also connected to the culture medium 84, and the microcylinder 98 is connected to the inoculum 85. The supply means 48 includes an injector 99 comprising two microcylinders 100 and 101, which connect to the supply line 95. The microcylinder 101 also connects to the supply of water 86.

When a test-tube T is at supply station I, and while the cap 23 is being removed therefrom, the arm 87 is raised and is at position C, above a container 90, which holds sample solution 92 and is covered by a sheet of aluminium foil 93. The cylinder 81 and plunger 82 assembly is actuated to retract the plunger 82, and lower the arm 87. Hereupon, the longer nozzle 89 pierces the aluminium foil 93 and the lower end thereof enters the sample solution 92. The microcylinders 100 and 101 are now actuated independently, the microcylinder 100 drawing in a required amount of sample solution 92, and the microcylinder 101 similarly drawing in water 86. At the same time the cylinder 97 and microcylinder 98 also are actuated independently, drawing in required amounts of culture medium 84 and inoculum 85, respectively. Next, the cylinder 81 and plunger 82 assembly is actuated to raise the plunger 82 and arm 87, thus withdrawing the nozzle 89 from the sample solution container 90, whereupon the sampling unit 91 moves the next container 90 into line. While the arm 87 is still raised, the rotary solenoid 83 is actuated, and the arm 87 is swung to position D. The plunger 82 is then retracted, to lower the arm 87, and bring the nozzles 88 and 89 into the mouth of the test-tube T. The contents of the microcylinder 100, and cylinder 101 are now injected together along the supply line 95, and the contents of the cylinder 97 and microcylinder 98 are injected together along the supply line 94, whereby the required amounts of sample solution 92, water 86, culture medium 84, and inoculum 85 are injected into the test-tube T. The arm 87 is then raised and swung back to position C, the cap 23 is replaced on the test-tube T, the test-tube T is moved forwards, and the next test-tube T is brought into line, and the above-described process is repeated.

To prevent contamination by external air during this injection process, the section containing sample solutions is further protected by a plastic cover, and also aseptic air supplied to the chamber 40 by the aseptic constant supply means 41 keeps the pressure inside the chamber 40 positive with respect to outside pressure. An aseptic atmosphere may be further ensured by the provision of glow-type germicide lamps, or gas means employing ethylene oxide for eliminating bacteria, or similar means.

As shown in FIGS. 14 and 15 the above-mentioned test-tube holding means 49 in the assessment station II comprises an electrically driven cylinder 102 and plunger 103 assembly, a clamp shift element 104, clamps 105, and a fixed stand 106.

The fixed stand 106 comprises a top plate 106*a* and a lower plate 106*b*, both of which are horizontal and supported by vertical supports 106*c*. Two elliptical openings 107 are formed in the top plate 106*a*. The major axes of the openings 107 lie on the same line. Two corresponding elliptical openings 107 are formed in the lower plate 106*b*, in line with the openings 107 in the top plate 106*a*. Each clamp 105 comprises an upper, semicircular portion 105*a* and a lower, rod portion 105*b*. Each clamp rod portion 105*b* passes through the fixed stand top and lower plates 106*a* and 106*b*, and fits slidably in a pair of openings 107 formed therein. The lower end of each portion 105*b* extends to below the lower plate 106*b*, and a retainer ring 108 is attached thereto, to prevent the clamp 105 being slid upwards out of the fixed stand 106. When the clamps 105 are thus slidably mounted in the stand 106, the lower ends of the clamp semicircular portions 105*a* rise slidably on the upper surface of the fixed stand top plate 106*a*, and the semicircular portions 105*a* lie opposite, and are curved towards one another. In each clamp semicircular portion 105*a* there is formed an opening 109, which permits passage of light for turbidimetric determination of bacterial growth in test-tube T, as described below. The clamps 105 may be moved towards or away from one another by the sliding motion of the rod portions 105*b* in the openings 107.

The clamp shift element 104 has a substantially trapezoidal shape, and has two longitudinal guide slots 110 which are inclined at an angle to one another, whereby the guide slots 110 form a general V shape. The clamp shift element 104 slides freely between the top and lower plates 106*a* and 106*b* of the fixed stand 106, and in this configuration the clamp rod portions 105*b* lie within the guide slots 110. When therefore the clamp shift element 104 is pushed towards the fixed stand 106, and the narrow end of the V formed by the guide slots 110 approaches the stand 106, the clamp rod portions 105*b* are compelled to move along the guide slots 110, and also along the openings 107, towards one another, that is, the clamps 105 are brought together. Contrariwise, when the clamp shift element 110 is pulled away from the stand 106, the clamps 105 are moved apart.

The clamp shift element 104 is caused to move towards or away from the stand 106 by actuation of the electrically driven cylinder 102 and plunger 103 assembly, the line of action of which is generally parallel to the guide rail 39. Opposite ends of a connecting shaft

111 are fixedly attached to the plunger 103 and to the narrow end of the shift element 104, respectively. Outward or inward movement of the plunger 103 with respect to the cylinder 102 therefore causes the shift element 104 to move towards or away from the stand 106, and the clamps 105 to move together or apart. As described earlier, the assessment station II is located near the lower end of the outer side of the third guide rail loop 39*c*, and below the reciprocating bar short section 34*c*. When a test-tube T transported on the guide rail 39 travels down the outer side of the third loop 34*c*, and past the short bar section 34*c*, it comes to a position above the fixed stand 106 and between the clamps 105. The cylinder 102 and plunger 103 assembly is thereupon actuated to cause the plunger 103 to move outwards, the clamps 105 are moved together, and the clamp semicircular portions 105*a* fit around, and secure the test-tube T in a fixed position. When the test-tube T is thus secured it still remains in its holder 35.

The above-mentioned electrophotometer 50 comprises a light source 112, lens assembly 113, and slit board 114, which are located on one side of the fixed stand 106, and a photoelectric cell 116, which is located on the opposite side of the fixed stand 106. The lens assembly 113 is situated between the light source 112 and the stand 106, and the principal focal plane thereof lies generally in line with the center of the stand 106, that is, where a test-tube T is held. The board 114 has a slit 115 formed therein, to permit passage of light from the light source 112, and is provided within the lens assembly 113. When a test-tube T is held in the clamps 105, as described above, light from the light source 112 is directed by the lens assembly 113 and slit 115 through the opening 109 in one clamp 105*a*, through the culture contained in the test-tube T, through the opening 109 in the other clamp 105*a*, and onto the photoelectric cell 116. The optical density of a culture is proportional to the growth of bacteria therein, and the value obtained is recorded immediately in the recording unit 51. There is, of course, no need for intervention of staff to determine and record this value, but the whole process is effected automatically by suitable electrical connections between the photoelectric cell 116 and recording unit 51.

When this process is completed, the plunger 103 is drawn in, the clamp shift element 104 is drawn away from the stand 106, and the clamps 105 release the test-tube T. The test-tube T is now pushed forward by the next test-tube T moving down the outer side of the 3rd loop 39*c*, after which this next test-tube T is held in the clamps 105 and the optical density of the light by the culture therein determined and recorded, as described above. Alternatively, the first test-tube T may be removed. In either case, succeeding test-tubes T are brought to the assessment station II and the growth of the cultures therein determined and recorded.

Thus, according to the present invention, testtubes T containing bacterial cultures are held in holders 35 and advanced one step at a time by drive means 42 and fast advance means 43 along a guide-rail 39 in a chamber 40 wherein an aseptic atmosphere at constant temperature is maintained by the means 41. The length of the guide rail 39 is adjustable, and while test-tubes T are transported thereon they may be shaken by reciprocating long bar 34. Successive test-tubes T are brought to a supply station I, where culture medium sample solution and inoculum are automatically supplied thereto, and are subsequently brought to an assessment station II, where the bacterial growth in culture therein is automatically determined and recorded.

Experiments were carried out to assess the operational efficiency of the method and means of the invention.

EXPERIMENT III

200 test-tubes, having dimensions of 15 × 150 mm, and a weight of 15.5 ± 0.2 g were employed. An O-ring was provided around each test tube at the site of 115 mm for the bottom thereof 3 ml of a culture medium, having a composition comprising 20% sorbitol, 1% corn steep liquor and 0.03% calcium carbonate, were inserted in each test-tube, and protected by a rubber cap, having dimensions of 20 × 47 mm, and a weight of 6.5 g, covering the test-tube. The culture medium in each test-tube was innoculated with Acetobactor suboxidans, and the experiment proceeded with the production of sorbose, at 30°C, for 24 hours, during which time the test-tubes were shaken by a reciprocating long bar striking the bottoms thereof, the impact frequency being 150 cycles per minute (cpm), and the bar having a stroke length of 40 mm. For the purposes of comparison, conventional shaking equipment, having an impact frequency of 140 cpm, and a stroke length of 35 mm, was employed for the production of sorbose under the same conditions, that is, at 30°C for 24 hours. The results of the experiment show that whereas the progression rate of sorbose production from sorbitol using conventional equipment was only 40%, the average production rate attained with the means of the invention was 80%, thus demonstrating the marked superiority of the means of the invention. It is also to be noted that the coefficient of variation from this average value for 200 samples was only ± 3%, in other words the rate of sorbose production is extremely even and reliable.

EXPERIMENT IV

There were employed test-tubes T having dimensions of 15 × 150 mm, and being of equal thickness. The empty test-tubes were covered with rubber caps, and after sterilization were suspended in holders in an aseptic, constant-temperature chamber, which was set to 37°C. L-isoleucine solutions varying from 0 to 150 μg/ml in concentration were pre-loaded into sample solution containers, the samples being divided into 8 levels of 10 samples each. The containers were covered with aluminum foil, and, after sterilization, were mounted in a sampling unit. There was also prepared water for dilution of samples and a suspension of Leuconostoc mesenteroides in the medium for isoleucine assay. Operation then commenced, 0.1 ml of the sample solution being drawn into one microcylinder of one injector and 1.4 ml of water being drawn into the other cylinder thereof, while at the same time 1.5 ml of the culture medium containing the inoculum suspension was drawn into the cylinder of another injector. Successive test-tubes were brought into line with the injector nozzles, the caps removed therefrom, the above-described quantities of sample solution, culture medium and inoculum injected there into, and then the caps were replaced thereon. The test-tubes were slid onto the guide rail at 30 second intervals, and then left stationary, the cultures therein being allowed to incubate for approximately 24 hours. After this period, the test-tubes were shaken by the shaking having the short bar means to produce a uniform suspension of bacteria,

and then the test-tubes were brought one at a time to the assessment station, where they were held stationary and the optical density at 660 mμ was measured. The results obtained are shown in the graph of FIG. **16**, in which, for comparison, are also plotted the results obtained by using a conventional manual method.

The results show that a concentrations of 10 and 20 μ g/ml, the coefficient of variations with the conventional method were ± 1.3 and ± 0.8, respectively, whereas the variations using the means of the invention were only ± 0.6 and ± 0.4.

As is clear from the above description, the present invention provides a method and means wherein the whole process, from injection of sample solution, culture medium, and inoculum into test tubes to the repeated measurement of bacteria growth in the cultures, can be carried out automatically with improved accuracy. The invention also offers the advantages of improved efficiency and economy of space by carrying test-tubes containing cultures over a zig-zag path. The construction of the zig-zag guide rail supporting the test-tubes makes it possible to adjust the length of the path over which the test-tubes are carried, and so to adjust the number of test-tubes dealt with, and vary to the duration of the process. The degree of shaking of test tubes is easily adjustable by varying the impact frequency or the stroke length of the reciprocating bar which strikes the test-tubes containing the cultures, or by adjusting the setting of the test-tubes in the holders upwards or downwards. The method and means of the invention also offer great advantages over conventional methods and means with regards to the measurement of bacterial growth in the cultures. With conventional methods and means, it is necessary to transfer the culture of each test tube into the cell in an electrophotometer. There is a possibility of contamination with other cultures during this transfer process. Conventional methods and means have the further disadvantages that determination of bacterial growth by the turbidimetric method can be effected only once for each culture. In contrast to this, with the method and means of the invention, the optional density of cultures may be determined without transfer of the cultures into the cells, and the determination is therefore simple and may be carried out repeatedly if so required. The invention also offers the advantage that portions of the cultures may be withdrawn at any stage in the incubation thereof, reversing the action of the injection nozzles at the supply station.

What is claimed is:

1. A method for bacterial culture wherein sample solution, culture medium and inoculum are placed in a plurality of test tubes, said test tubes are suspended from a horizontal axis above the center of gravity thereof, and said test tubes are cyclically struck by a reciprocating bar acting on a certain level, whereby an oscillatory motion is imparted to said test tubes, and said test tubes and the contents thereof are shaken.

2. A method as claimed in claim **1** wherein the point at which the test tubes are struck and the frequency of impact is such that the period of the oscillatory motion of the test tubes is half the frequency of impact and the amount of oscillatory motion is no greater than 50° from the vertical.

3. A method for continuous bacterial culture and the determination of the growth thereof, wherein sample solution, culture medium, and inoculum are automatically supplied to test tubes freely suspended inholders which are rotatable around a horizontal axis, the holders being supported by and slidable on a guide rail within an aseptic, constant temperature chamber, sliding said holders holding said test tubes around said guide rail by suitable drive means, cyclically striking said test tubes by striking there against at least one bar by moving the bar reciprocally on a certain level for imparting an oscillatory motion to said test tubes and shaking the contents thereof, and repeatedly determining the bacterial growth in said test tubes at a certain location along said guide rail without removal of said holders from said guide rail or removal of said contents from said test tubes.

4. An apparatus for bacterial cultures by shaking comprising a support means, a frame mounted on said support means for free adjustment upwards, downwards, fowards and rearwards relative to the support means, test tube holders mounted on said frame for oscillating movement around a horizontal axis in said frame, for holding test tubes suspended therefrom which test tubes contain sample solution, culture medium and inoculum to be incubated, whereby said test tubes may oscillate freely about the points of support of said holders, a drive means and a bar connected to said drive means and driven in a continuous reciprocating motion towards and away from the positions of the test tubes suspended in said holders for cyclically striking said test tubes, whereby the test tubes are caused to oscillate and contents thereof are shaken.

5. An apparatus for continuous bacterial culture comprising a zig-zag guide rail, an aseptic constant temperature chamber within which said guide rail is mounted, test tube holders slidably positioned on said guide rail, said holders being oscillatable around a horizontal axis for permitting test tubes suspended therefrom to oscillate freely, advance means provided at suitable locations adjacent to said guide rail for advancing said holders along said guide rail one at a time and at regular intervals, means adjacent one point along said guide rail for automatic supply of sample solution and culture medium to said test tubes and for injection of said sample solution and culture medium with inoculum, a holding station at a further point along said guide rail having means for holding test tubes stationary and determining the bacterial growth in the test tubes without removal of said contents from said test tubes, bars extending parallel to certain portions of said guide rail, and drive means connected to said bars for driving said bars in a reciprocating motion along a certain level for cyclically striking test tubes freely suspended in said holders along said guide rail, whereby contents of said test tubes are shaken and bacterial growth therein is accelerated.

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