This invention relates to mechanized apparatus for rapidly and accurately filling a series of containers with a measured quantity of liquid and/or for withdrawing a measured volume of liquid from one container, transferring it to a succeeding container, mixing the transferred liquid with another liquid already present in such container and withdrawing therefrom a measured volume of the mixed liquid, the apparatus being adapted to repeat this sequence through a series of successive containers. More particularly, this invention relates to an improved automatic motor-driven machine for performing multiple serial dilutions.

In such fields as immunology, microbiology and virology, one of the most time consuming laboratory operations is the manual performance of serial dilutions used in test procedures such as metabolic inhibition tests for antibodies to polio virus wherein serial dilutions are used for titrations of the antipolio virus activity in the polio serum.

The manual preparation of a serial dilution for such tests consists of first dispensing a predetermined volume of concentrated liquid serum from a syringe or mouth pipette into the first of a series of test tubes. The remaining test tubes are filled with equal measured amounts of a diluent, such as a balanced salt solution with pH indicator. A serial dilution is then made by withdrawing a measured amount of the sample from the first test tube by means of a manual syringe or pipette, transferring it to the second test tube, dispensing it into and then mixing it with the diluent in the second test tube by pumping the syringe several times, and then withdrawing the same measured amount of the diluted serum from the second test tube and repeating the test tube wherein the procedure is repeated. After this procedure has been repeated successively through the remaining test tubes, a progressively weaker concentration of serum solution is present in each successive test tube of the series.

Polio virus is then manually added in equal amounts to each test tube, followed by the culture medium which contains monkey kidney cells. Virus activity and its inhibition in each test tube are indicated by color changes or cell destruction in the culture medium. The dilution to which the serum can be diluted and prevent the virus from inhibiting the cells’ metabolism provides an assay of the antibody level in the serum.

An object of the present invention is to provide an improved automatic machine for performing serial dilutions or analogous liquid handling operations, thereby saving valuable man hours and virtually eliminating the factor of human error.

Another object is to provide an automatic machine adapted to perform both filling and serial dilution procedures in a rapid, accurate and more sterile manner.

A further object of the invention is to provide a machine of the above character which is easy to operate and clean, and which is readily adjustable as to the quantity of liquid handled as well as to different sizes and types of containers.

Other objects, features and advantages of the invention will become apparent from the following description taken in conjunction with the accompanying drawings in which:

FIG. 1 is a side elevation of a serial dilution machine constructed in accordance with the present invention illustrating a test tube rack at the end of the diluting cycle with the pipettes raised above the last row of test tubes, one portion being broken away to illustrate detail.

FIG. 2 is a fragmentary vertical section taken on the line 2--2 of FIG. 7, illustrating the pipettes lowered into the test tubes.

FIG. 3 is an enlarged fragmentary vertical section taken on the line 3--3 of FIG. 6 with a portion broken away to illustrate detail.

FIG. 4 is an enlarged fragmentary vertical section taken on line 4--4 of FIG. 7.

FIG. 5 is a further enlarged fragmentary vertical section taken on the line 5--5 of FIG. 6.

FIG. 6 is an enlarged fragmentary and elevation looking towards the right side of the machine as viewed in FIG. 1.

FIG. 7 is an enlarged fragmentary horizontal section taken on the line 7--7 of FIG. 6.

FIG. 8 is a fragmentary and elevation illustrating a cross row of test tubes spaced more closely together than the arrangement of FIG. 6.

FIG. 9 is a fragmentary plan view of the syringe clamp structure illustrating smaller syringes than those shown in FIG. 7 and more closely spaced to accommodate the test tube arrangement of FIG. 8.

FIG. 10 is a fragmentary side elevation, partially schematic, of a syringe-pipette assembly converted to filling operation, portions being broken away to illustrate detail.

FIG. 11 is a fragmentary side elevation of a filling cam and associated cam follower.

FIG. 12 is a perspective view of an interchangeable cam follower for use with either the diluting or filling cams.

FIG. 13 is a fragmentary side elevation of a filling head attachment also in accordance with the present invention which may be used in lieu of the filling conversion apparatus of FIGS. 10 and 11, the filling head being shown in FIG. 13 at the lower limit of its travel.

FIG. 14 is a fragmentary sectional view taken on the line 14--14 of FIG. 15, but with the filling head shown at its upper limit of travel.

FIG. 15 is a front elevation of the filling head taken partially in section along line 15--15 of FIG. 13 with a portion of the filling attachment apparatus shown schematically.

General arrangement and operation

Referring generally to FIG. 1, the serial dilution machine of the present invention includes a row of automatically operated pump assemblies each comprising a syringe 20 and pipette 22 mounted in a vertically movable holder assembly, indicated generally by the reference numeral 24, which surmounts a flatbed carriage 26 and is horizontally movable in a direction transversely of the row of syringes 20. Carriage 26 accommodates a group of suitable containers such as a rack 28 of test tubes 30 arranged in one or more rows aligned with the direction of carriage travel and aligned transversely in cross rows for making multiple serial dilutions. The syringes 20 operate in unison over each lengthwise row of test tubes for up to twenty (for example) successive dilutions. The carriage has a positive positioning device that insures placement of each successive container directly under the pipette tips. Holder 24 carries a pumping head indicated generally at 32 which moves vertically relative to holder 24 to operate the syringe plungers 34 in timed relation with the holder and carriage movements.

The general operation of the machine as a serial dilutor is as follows: Assuming that a twofold serial dilution of four lengthwise rows of ten test tubes each is
to be performed, a predetermined volume of sample to be diluted is dispensed into each tube of the first cross row 31 of test tubes 30 and the remaining test tubes are each filled with 1/2 of this volume of diluent. Preferably, the filling is done simultaneously with the serial diluting by using the filling head attachment of FIGS. 13-15, described later, but it may also be done by converting the machine to filling operation using the apparatus shown in FIGS. 10 and 11, or manually. Assuming that the test tubes have been filled manually, rack 26 is placed on carriage 26 and the carriage is moved to the broken line position shown in FIG. 1 wherein the first or sample cross row 31 of test tubes is looing downward the raised pipettes 22. The machine is started and automatically cycles through the following movements: (1) pipettes 22 are lowered into the sample in the first row of test tubes; (2) the plungers of syringes 26 are reciprocated five times to mix, and on the final upstroke, to aspirate 1/2 of the sample into the associated pipette; (3) pipettes 22, with the aspirated samples therein, are raised clear of the first row of test tubes; (4) carriage 26 indexes to bring the second row of test tubes directly beneath the pipettes. The foregoing cycle is repeated several times so that the sample is first pumped from the pipette into the diluent and then mixed with the diluent during the five-stroke pumping action so that a half strength serum is made up in the second row of test tubes. On the final upstroke one half of this half strength solution is withdrawn into the pipette for transfer to the third cross row of test tubes where the discharge and mixing action is repeated to produce a quarter strength solution in the third row. This sequence is repeated on down the line of test tubes until the last dilution has been made. The machine then withdraws the pipettes from the last row of tubes and shuts itself off.

The illustrated embodiment of the machine is designed to accommodate any rack of test tubes, capsules, plates or plastic dilution trays, etc. within the maximum dimensional limits of the carriage. Holder 24 is designed for interchangeability of different size commercially available syringes 26, such as 1, 2, 5 and 10 cc., as well as for different lengths of commercial pipettes. Any desired dilution factor can be obtained by regulation of the volume of the diluent and the volume aspirated.

The machine can also be used to pump diluent into the test tubes prior to operating the machine as a serial dilutor by (1) connecting a two-way valve 38 (FIG. 10) in each syringe-pipette assembly, (2) connecting the valve inlet to a common source 40 of diluent and (3) converting the pumping head to single stroke action by changing to a single lobed filling cam 42 (FIG. 11). Alternatively, one cross row of empty test tubes may be filled with diluent by the filling head attachment of FIGS. 13-15 while the next successive cross row of filled tubes undergoes serial dilution, thereby further reducing the total time and labor involved in obtaining serial dilutions.

**Framework**

The serial dilution machine of the invention includes a horizontal platform 44 (FIGS. 1 and 6) which supports carriage bed 36 as well as an upright column 46 (FIGS. 6 and 7) located to one side of the bed. A sleeve 48, mounted for vertical movement on column 46, is raised and lowered by a worm and worm wheel arrangement 50 which threadably engages a traveling nut 51 (FIG. 7) which is bolted to the sleeve and extends into column 46 through a vertical slot therein. Sleeve 48 is clamped at the desired elevation by a clamp screw 52 and supports pump platform 54 which extends laterally therefrom over bed 36 (FIG. 6). Platform 54 supports the holder assembly 24, pumping head 32, associated parts and drive mechanism therefor. A pair of laterally spaced upright posts 56 are rigidly supported by blocks 58 welded to platform 54 (FIGS. 3 and 7). Each of these posts slidably supports a lower guide block 60 which carries holder assembly 24 and an upper guide block 62 which carries the pumping head assembly 32.

Platform 54 also supports a pair of spaced upright plates 64 and 66 (FIG. 7) in which a camshaft 68 is journaled for rotation by a pulley 70 affixed to one end thereof and connected by a timing belt 72 to the output pulley 74 (FIG. 1) of an electric motor and gear reduction drive unit 76. The drive unit 76 is attached to a bracket 76' carried by platform 54 and sleeve 48.

**Carriage and associated operating mechanism**

Carriage 26 comprises a rectangular frame 77 surrounding a bed plate 78 (FIG. 6) which rides on ways 80 of bed 36. Four locating blocks 82 adjacent the respective corners of rack 28 locate it properly on plate 78. A rack bar 84 secured to the underside of the inner side member of frame 77 (FIG. 6) is engaged by a spring biased pawl 85 pivoted on a sliding block 88 (FIG. 1). Block 88 is reciprocated by a bellcrank linkage comprising a connector 90 pivoted on block 88, a rod 92 interconnecting one end of a bellcrank 94 with connector 90, an adjustable link 96 connected between the other end of the bellcrank 94 which is fastened at 100 in a yoke 102 carried by platform 54. The other end of lever 98 carries a cam follower roller 104 which rides against the periphery of a single lobe cam 106 secured camshaft 68 for rotation therewith adjacent pulley 70.

The lobe of cam 106 depresses the follower end of lever 98 once during each revolution of camshaft 68 which rocks bellcrank 94 from the dotted to solid line positions of FIG. 1 to thereby advance block 88 and pawl 86 and hence the carriage by a predetermined amount corresponding to the lengthwise spacing between adjacent cross rows of test tubes. When cam follower 104 returns to the base circle of cam 106 under the influence of the weight of the linkage, pawl 86 is retracted so as to engage the next tooth of rack 84.

**Syringe-pipette holder assembly and associated operating mechanism**

Each syringe 29 and associated pipette 22 is clamped together by holder assembly 24 for vertical reciprocating movement as a unit along posts 56. Holder 24 comprises a plate 110 (FIG. 3) secured to blocks 60 which supports a rectangular frame 112 having a front rail 114 and a rear rail 118 fastened to plate 110 by thumb screws 116. A series of double-ended syringe clamp blocks 120, one for each syringe, are hung on rail 118 and removabley secured thereto by ball plungers 121, one end of which has a large radius clamping surface 122 (FIG. 7) for engaging the upper barrell of larger diameter syringes, and the other end having a smaller radius clamping face 124 which, by reversing clamp 120, can be used for smaller diameter syringes. Rail 114 supports a three piece syringe clamp comprising a hanger 126, a clamping member 128 and a thumb screw 130 threaded in hanger 126 for developing clamping pressure against the syringes.

The machine is designed to use commercially available 1, 2, 5 and 10 cc. syringes (such as those sold under the trademark "Cornwall" by Becton, Dickinson & Co.) with metal handles removed, although other suitable syringes may be substituted if desired. Each syringe includes the spring-biased metal plunger 34 which extends from the upper end thereof and a quarter turn faster tip, such as that known commercially by the trade mark "Luer-Lok," at the lower end. This tip includes a male part 132 (FIG. 5) having a tapered needle which seats with a seal fit in a female part 134 which is quick threaded in the male part 132.

The pipette clamping structure of holder assembly 24 includes a pair of journal blocks 136 (FIG. 3) welded to plate 110 adjacent the side edges thereof (FIG. 6), a
hanger 138 secured to each block 136 by thumb screws 140 and notched to engage the bottom edge of plate 110, a clamp bar 142 secured at its ends to hangers 138, and a series of notched pipette sealing blocks 144 (FIG. 5), one for each syringe-pipette assembly, which are removable from the syringe 112 by thumb screws 146 threaded in bar 142. The female fastener tip 134 is brazed in the upper end of a bore 148 in block 144, and the brazing material 149 as well as the lower end of tip 134 are machined flush with the lower end of bore 148. This machined surface provides a stop for a rubber sealing sleeve 150 which is inserted in the upper end of a larger co-axial counterbore 152. Counterbore 152 also slidably receives a metal sleeve 154 which extends through the bottom of a U-shaped saddle 156 and is secured there- to by a retaining ring 158. The upper end of pipette 22 is slidably inserted up through sleeve 154, past an O-ring retainer 160 therein, and through sealing sleeve 159 until it abuts material 149. Pipette 22 is removably retained in this position by O-ring 160 until clamping pressure is applied to the pipette by drawing saddle 156 up- wardly relative to block 144. This forces sleeve 154 up against sealing sleeve 150, compressing the latter so that it grips the metal sleeve 152 of pipette 22 and also seals the space between pipette 22 and counterbore 152. In addi- tion to retaining the pipette when it is unclamped, O-ring 160 co-operates with sealing sleeve 150 to maintain the vertical orientation and alignment of the pipettes.

Each line of a diluting cam 200 (FIG. 4) having five lobes 202 and a cam follower roller 204 attached to one arm 206 on a bellcrank lever 207 which is fulcrumed on a pivot pin 208 extending through a spacer 210 and secured in an upright plate 212 mounted on one of the guide blocks 60. The other arm 214 of lever 207 has a Z-shaped member 216 fastened to the underside thereof to form a forked end which receives a roller 218 journalled on the lower end of a slotted plate 220 which is adjustably secured by four studs 222 to the side of guide block 62. A tension spring 224 is secured at one end to an eccentric boss 226 of a tension adjusting disc 228 rotatably mounted on the outer upper corner of plate 212, and is secured at the other end to lever member 216 so as to bias cam follower 204 into engagement with cam 200.

As shown in FIG. 1, when holder assembly 24 is raised to its uppermost position by lever 174, pumping head 32 likewise moves upwardly with holder 24 since lever 207 is carried by plate 212 which moves with lower guide blocks 60. In this position, cam follower 204 is raised clear of cam 200, but lever 207 is maintained in raised position by spring 224 which holds the lever against an adjustable stop 230, thereby supporting pumping head 32 in its fully raised position while follower 204 is dis- engaged from cam 200. Cam 200 is synchronized with cam 182 so that holder assembly 24 is lowered just prior to, and is raised immediately after, the pumping period in which the five lobes 202 of cam 200 successively en- gage follower 204. During this pumping period the lobes pivot lever 207 up and down five times while the pipettes 22 are immersed in the liquid in test tubes 30, thereby vertically reciprocating pumping head 32 five times relative to holder 24 so that plungers 34 are like- wise stroked between the raised and lowered positions thereof shown in dotted and solid lines respectively in FIG. 4.

Assuming that pipettes 22 have just been lowered into the sample cross row of test tubes at the beginning of the diluting cycle, the first downstroke of syringe plunger 34 causes air to be expelled from the syringe barrel downwardly through pipette 22 and into the liquid sample in the test tube. On the first upstroke of plunger 34 the liquid is drawn into pipette 22, and on the second down- stroke is again expelled into the test tube. This alter- nating intake and discharge of the sample from the pipette continues through the remainder of the five pumping strokes, the last stroke being an upstroke of plunger 34.

Pumping head assembly and associated operating mechanism

Pumping head assembly 32 includes guide blocks 62 which support a horizontal rail 190 (FIGS. 3 and 6) notched along its outer edge to slidably receive endwise thereon a series of plunger operators 192, one for each syringe plunger 34, which are adjustably secured along rail 190 by thumb screws 194. Plunger operators 192 each comprise a metal block adapted to overhang the upper end of the associated plunger 34. A headed pin 196 is mounted for limited vertical movement in each operator 192 and is biased downwardly by a spring 197 so as to normally protrude beneath operator 192 for yieldable engagement with a key 198 affixed to the upper end of each plunger 34. Springs 197 are stronger than the syringe springs (not shown) and urge plunger 34 upwardly so that pins 196 do not normally yield during engagement with keys 198 until after the associated plungers have bottomed in their respective syringes. The spring-loaded pins 196 insure full-stroke actuation of a group of syringe plungers 34 in performing multiple serial dilutions, this compensating for the relatively large variations in the length of commercially available plungers 34, measured from the lower end of the plunger 34 to the top of keys 198. Accuracy in both the diluting and filling operations requires that all of the plungers 34 bottom against the lower end of their respective syringe barrels. This is insured by the yieldable or lost motion connection afforded by pins 196 without thereby jamming an over-length plunger against the bot- tom of the syringe barrel with such force as to damage either the syringe or plunger. On the upstroke of pumping head 32, the head continues to travel upwardly for a short distance beyond the upper end of each of the plungers 34 to reach their upper limit as determined by the setting of the threaded adjusting stem 199 of the syringe.
to draw the predetermined measured quantity of sample into pipette 22. Syringe plunger 34 is biased to its raised position by a spring in the syringe (not shown) on the upstroke of the pumping head, and the syringe spring maintains plunger 34 in this position when pipettes 22 are being raised from and lowered into the test tubes.

After pipettes 22 clear the upper ends of the sample test tubes, carriage 26 indexes to bring the second cross row of test tubes beneath pipettes 22, and then cam 182 lowers holder 24 to drop pipettes 22 into the second row of test tubes, simultaneously bringing cam follower 204 back into engagement with the base circle of diluting cam 208. The five pumping strokes are again repeated so that the measured portion of sample withdrawn from the sample test tubes is first expelled into the diluent in the second row of test tubes and then the mixture of sample and diluent is alternately drawn into and expelled from the pipettes to obtain thorough mixing and dilution to half strength in the second row of test tubes. On the last upstroke of the syringe plunger, the same measured amount of the half strength solution in the second test tube is drawn into pipettes 22 for transfer to the third row of test tubes, and so on throughout the cycle.

**Conversion to filling machine operation**

The above described serial dilution machine may be readily converted in the following manner for use in filling the second and successive cross rows of test tubes with equal amounts of diluent preparatory to making a serial dilution. Pipette clamping lever 172 is rotated so as to drop saddles 156 to their lowermost position, thereby relieving pressure on sealing sleeves 159. Pipettes 22 are then removed from sealing blocks 144. Next, syringe clamps 128 are loosened and syringes 20 are disconnected from blocks 144 and removed from holder 24. Blocks 144 are then removed from sleeves 154, clamp block bar 142 also being removed in this procedure.

Then, as shown in FIG. 10, a commercially available valve-cannula assembly, comprising a two-way valve fitting 38 having lower and upper Luer-Lok fasteners 132 and 134 at the respective ends of the vertical branch thereof and having a hollow stem (cannula) 240 secured to the lower fastener 132, is connected to the lower male Luer-Lok fastener tips 132 of syringe 20. Exchangeable 38 has one end of a conduit 242 connected to the T inlet thereof, and the other end of each conduit 242 is immersed in the common supply flask 40 of diluent. Each syringe-valve-cannula assembly is inverted while removed from holder 24 and stroked a few times to prime it with diluent and to get rid of any air in the system. Then each assembly is mounted in holder 24 by inserting stem 240 downwardly through sleeve 154 until the upper end of stem 240 seats on the upper end of the sleeve, and then reclamping the syringes.

Pumping head 32 is converted from five-stroke diluting operation to single stroke filling operation by detaching the supporting block 244 for cam follower 204 (FIGS. 9 and 12) from the end of lever arm 206, turning it over, and re-attaching it to the arm so as to position follower 204 in tracking relation with the single lobe filling cam 42 as shown in FIG. 11 and as indicated in phantom in FIG. 7. The single lobe 2/6 of cam 42 is positioned relative to cam 182 so that syringe plungers 34 are depressed by operators 192 and raised by the syringe springs once during each dwell period of cam 182, that is, after cannulas 240 are lowered into the test tubes and prior to their removal therefrom.

The machine cycles automatically in the same manner as previously described except that there is only one plunger reciprocation per test tube and liquid is not withdrawn from one test tube for transfer to the next successive test tube. On the downstroke of plunger 34, inlet check valve 249 closes and outlet check valve 250 is opened by the pressure of diluent therefrom so that the predetermined quantity of diluent is dispensed into the test tube. On the upstroke of plunger 34, valve 250 closes and valve 249 opens to admit the predetermined quantity of diluent to the syringe chamber. After a series of test tubes, have been filled with an equal amount of diluent, the machine automatically shuts itself off with the cannulas raised clear of the test tubes.

The above conversion procedure is reversed in order to reconvert to serial dilution operation.

**Controls**

The invention disclosed herein relates to the structure and arrangement of mechanical components as exemplified by the above-described machine and therefore a detailed disclosure of the electrical controls and circuitry has been omitted for the sake of brevity. However, it is to be understood that conventional control equipment may be employed to start and stop the electric motor of drive mechanism 76. Suitable limit switches 252 and 254 (FIG. 1) are mounted on bed 44, switch 252 being closed by engagement with carriage 26 when it is moved to the stop position shown in broken lines in FIG. 1. This connects a cycle start switch branch circuit between the motor and power source so that when the cycle start switch is pushed the motor is energized and initiates the mechanical motions previously described. When the carriage strikes limit switch 254 at the end of its travel, the motor energizing circuit is switched off by limit switch 256 mounted on platform 54 beneath a switch 308 acting on a limit switch 256 which is secured to the end of camshaft 68 for rotation therethrough. The motor continues to run until cam 258 opens limit switch 256, this event being timed to occur after the pipettes have been raised to their uppermost position.

Conventional emergency stop controls as well as run/jog controls are provided to facilitate set-up and adjustments.

**Modified filling apparatus**

In lieu of converting the above-described serial dilution machine to filling operation as previously described, both filling and serial dilution may be performed at the same time by providing the modified filling apparatus shown in FIGS. 13, 14 and 15, also in accordance with the present invention. This apparatus in the form illustrated comprises a filling head attachment which is detachably mounted on holder 24 for movement therewith. Referring to FIGS. 13 and 14, the filling head comprises a block 300 having a pair of arms 302 secured by screws 304 to the side of plate 110 opposite that carrying the syringe-pipeet assembly. Block 300 supports a series of vertical filling cannula assemblies 306, one for each lengthwise row of test tubes, which are individually aligned in the direction of test tube travel with pipette 32. Cannulas 306 are spaced ahead of pipettes 22 by a predetermined distance so that cannulas 306 register with the cross row of test tubes immediately preceding the cross row of test tubes which register with pipettes 22.

As best seen in FIG. 14, block 300 has a throughbore 306 and three parallel blind bores 310, 312 and 314, bore 306 receiving a rotary metering and dispensing valve shaft 316 with a liquid seal fit therein, while bores 314, 310 and 312 serve respectively as diluent supply, excess diluent return, and fill passage. Shaft 316 has a series of radial passages 318, one for each filling cannula 306, having a predetermined volumetric capacity equal to the amount of diluent to be dispensed into each test tube during the filling operation. Shaft 316 is oscillated between the angular positions thereof shown in FIGS. 13 and 14 by a bifurcated operating lever 320 which is secured to the end of shaft 316 extending from the left side of block 300 as viewed in FIG. 15. The arms 322 of lever 320 received therebetween an operating pin 324 which is fixedly secured to block 38. Pin 324 preferably is an anti-friction type cam follower of known construction having an outer race supported
by needle bearings for rotation about a stud which is screwed into block 58.

As holder 24 reciprocates vertically to raise and lower pipettes 22 and filling cannulas 336 into and out of adjacent cross rows of test tubes, the movement of shaft 516 relative to pin 310 causes test tube 314 to swing about pivot 315 via line 330 and a pump 332 from a supply 334 flows upwardly through metering passages 318 to the return manifold 310 from which excess diluent is returned via line 336 to supply 334. During descent of holder 24 shaft 316 is rotated clockwise, causing the ends of passages 318 to move out of registry with ports 326, 328 so that the passages are sealed by the walls of bore 308, thereby trapping a measured quantity of diluent in passages 318.

When holder 24 reaches the lower limit of its downward travel, shaft 316 will have been rotated clockwise 13°, bringing test tubes 314 adjacent to metering passages 315 in register with ports 330 communicating with the air manifold bore 312 and with passages 340 communicating with the respective filling cannulas 306. Bore 312 is connected via line 342 to a constant low pressure air supply (not shown) and when passages 324 are brought into registry with ports 330 and passages 340, the diluent is rapidly expelled from each passage 318 into the associated test tube via the metering passage 340 and cannulas 306. Upward movement of holder 24 rotates shaft 316 counterclockwise, thereby shutting off the air to metering passages 318 and returning it to registration with the diluent supply and return ports 326 and 328.

The filling head attachment thus dispenses a measured quantity of diluent into the test tubes in proper synchronism with the movement of holder 24, and raises and lowers the cannulas 306 in proper synchronism with the test tubes in its indexing movement of carriage 26. The modified filling apparatus permits the filling operation to be performed simultaneously with the diluting operation, and reduces the manual tasks involved. The operator need only dispense the concentrated serum into the first cross row of test tubes, load the test tube rack on the carriage in the start position wherein the pipettes 22 are disposed above the first or sample cross row of test tubes and the cannulas 306 are disposed above the second, empty cross row of test tubes, push the start button and unload the rack which, because of the pitch of the rack itself, advances to the next cycle.

When filling cannulas 306 and pipettes 22 are spaced as shown for insertion into adjacent cross rows of test tubes, an extra cross row of test tubes may be provided at the trailing end of the rack to catch diluent while the pipettes are operating in what is actually the last cross row of tubes of the serially diluted series. However, it is to be understood that cannulas 306 and pipettes 22 may be arranged in closer proximity for insertion together into the same test tube, in which case the extra row of test tubes is not needed.

**Conclusion**

The above described serial dilution and filling machine in accordance with the present invention provides a highly versatile, accurate and rapid automatic mechanism which requires the part time attention of only one technician to perform multiple serial dilutions simultaneously. The lab technician is relieved of the time consuming and repetitive chore of making manual serial dilutions, and serial dilutions of much higher uniformity and accuracy are obtained. The machine is readily adjustable for various sizes of containers, and for various diameter syringes. The latter adjustment is obtained by shifting the syringe clamps laterally so that they are more closely spaced as shown in FIG. 9. Smaller, more closely spaced test tubes may then be used as shown in FIG. 8 to increase the output of the machine. The syringes and pipettes are also readily removable to facilitate cleaning and replacement thereof. The pipette clamping arrangement utilizing the resilient sealing sleeve 150 provides a perfect seal, permits quick removal and re-insertion of the pipettes and allows for a line-up adjustment to compensate for slight differences in the lengths of the pipettes.

**We claim:**

1. A machine for dispensing liquid into a series of containers including in combination, a carriage adapted to hold the containers arranged upright and in a row, means for moving said carriage in the direction of said row of containers, a support mounted adjacent the path of travel of the containers on said carriage, a pump-pipette assembly mounted on said support including a plunger and a pipette, said pipette extending downwardly from said support over the path of container travel for insertion into the containers, means for reciprocating said plunger and means for moving said pipette and said container thereunder vertically relative to one another for causing insertion and removal of said pipette into and from the container, said plunger reciprocating means being interconnected with said last-mentioned means for reciprocating said plunger during the time when said pipette is inserted in said container, said carriage moving means also being operably interconnected with said last-mentioned means to index the row of containers during the time when said pipette is removed from said container whereby in a repetitive cycle of operation the pipette is inserted into the first container in said row, the plunger reciprocated, the pipette withdrawn clear from said first container and the carriage moving means indexed to bring the next successive container beneath the withdrawn pipette.

2. The combination set forth in claim 1 wherein said support includes a holder for said pump-pipette assembly, said holder comprising a frame having a pair of rails spaced apart in the direction of container travel and extending transversely thereto, clamping means including a pair of members one supported on each of said rails opposite one another and adapted to receive the pump in clamped relation theretwixt, one of said members having a movable element connected thereto for adjustment towards and away from the other member for clamping and releasing said pump.

3. The combination set forth in claim 2 wherein said clamp members are notched to hang on said rails, said members frictionally engaging the rails when urged apart to clamp the pump and being slidable therealong upon release of clamping pressure, said clamping means in combination with the pump-pipette assembly of the row of containers on the carriage.

4. The combination set forth in claim 2 wherein the other one of said clamping members includes a pair of reversely positioned pump clamping means adapted respectively for clamping pumps of different sizes, and means for supporting said last-mentioned member on its respective rail with either of said portions in pump clamping position.

5. The combination set forth in claim 1 wherein said pump-pipette assembly includes a sealing block for interconnecting the pump and pipette of said pump-pipette assembly, said sealing block having means adapted for removable connection to one end of said pump and a bore communicating with said connecting means, a resilient sleeve in said bore fixed at one end against movement therein, said sleeve having an inside diameter dimensioned for slidably receiving said pipette in the relaxed condition of said sleeve, and means for compressing said sleeve axially towards said fixed end to reduce the inner diameter thereof and thereby clamp said pipette in sealed relation with said bore.

6. A machine for dispensing liquid into a series of containers including in combination transfer means adapted to hold the containers upright and arranged in a row,
means for moving said transfer means in the direction of said row of containers, a support extending over the path of container travel, a holder mounted on said support for vertical movement thereto, a syringe-pipette assembly removably mounted on said holder and including a syringe plunger and a pipette, said pipette extending downwardly from said holder for insertion into the containers, means for reciprocating the plunger of the syringe-pipette assembly and means for raising and lowering said holder, said plunger reciprocating means and said holder raising and lowering means being operably interconnected with said means for moving said transfer means to perform a repetitive cycle whereby the pipette is lowered into the first container in said row, the syringe plunger reciprocated, the pipette raised clear of the first container and the transfer means indexed to bring the next successive container beneath the raised pipette, this cycle being repeated throughout the row of containers.

7. The combination set forth in claim 6 including an upright support member on said support, said holder being slidably mounted on said support member for vertical movement therealong, said plunger extending vertically forward and said holder, said plunger reciprocating means respectively slidably mounted on said support member for vertical movement therealong above said holder for operating said plunger.

8. The combination set forth in claim 7 including means carried by said holder for vertically supporting said plunger reciprocating means so that the latter moves vertically with said holder.

9. The combination set forth in claim 6 wherein said respective means for reciprocating said plunger, raising and lowering said holder and moving said transfer means include a common shaft journaled on said support and having three cams therewith associated with said plunger, holder and transfer means, an electric motor for rotating said shaft, and a lever linkage associated with each of said cams for respectively operably interconnecting said holder, plunger, and transfer means with the associated cam, said cams being shaped and oriented relative to another and to their respective linkages to cause said holder, plunger and transfer means to operate in timed relation to cause said repetitive cycle of movements.

10. The combination set forth in claim 9 wherein said holder, plunger reciprocating means respectively include upper and lower guides slidably mounted for vertical movement on said support, a plunger operator mounted on said upper guide, said lever linkage for the plunger comprising a lever fulcrumed on said lower guide and having a supporting connection with said upper guide, said plunger having a cam follower mounted thereon adapted to operably engage the plunger cam only when said lower guide moves to its lowermost position.

11. The combination set forth in claim 10 wherein the plunger has a second cam in addition to said first-mentioned cam associated therewith, said first and second plunger cams being mounted on said shaft adjacent one another, one of said plunger cams being adapted to cause multiple reciprocation of said lever and the other of said plunger cams being adapted to cause a single reciprocation of said lever, said cam follower being adjustably mounted on said lever for optionally engaging either of said plunger cams whereby said machine may be operated for multiple stroke serial dilution or single stroke equal filling of the containers.

12. A machine for dispensing liquid into a series of open-end containers, said machine including in combination a horizontally bed, a carriage mounted on said bed for horizontal travel and adapted to hold the containers open-end up and arranged in a row aligned with carriage travel, an upright column mounted on said bed adjacent the path of travel of said carriage, a platform mounted on said column for vertical adjustment therelong and extending over the path of carriage travel, a holder adapted to support a plunger pump-pipette assembly and mounted on said plat-
prising a support extending over the path of container travel, a plurality of syringe-pipette assemblies mounted on said support, one for each row of containers, each assembly including a reciprocable syringe plunger and a pipette, said pipette extending downwardly from said support in alignment with the direction of travel of the containers in the row associated therewith, and a pumping head mounted on said support for travel in the direction of plunger reciprocation for simultaneously reciprocating all of said plungers, said pumping head having a plurality of plunger engaging members one aligned for engagement with each plunger and mounted for limited movement relative to said head in the direction of plunger reciprocation and a spring associated with each of said members for biasing the associated member towards the associated plunger, said pumping head being movable in the direction of plunger reciprocation to advance said members as a unit for engagement with said plungers to actuate the same through their respective pumping strokes, said members individually yielding against the force of said associated springs upon bottoming of said plunger and thereafter moving relative to the pumping head to accommodate differences in the respective strokes of said plungers.

17. A serial dilution machine comprising a rock for supporting a series of containers upright and in a row, a pipette and a filling conduit supported on said machine in fixed position relative to one another and arranged for insertion into the containers, horizontal drive means for moving said pipette and conduit relative to the containers horizontally in the direction of said row, vertical drive means for vertically reciprocating said pipette and conduit together relative to the containers, said horizontal and vertical drive means being operably interconnected to insert and withdraw said pipette and conduit into and from each of the containers successively, diluent supply means connected to said conduit and including valve means for causing a predetermined quantity of liquid diluent to be drawn into said conduit from the container in which it is inserted and pump means connected to said pipette for causing a predetermined quantity of liquid to be drawn into said pipette from the container in which it is inserted and in which the container is placed in the next succeeding container.

18. The combination set forth in claim 17 wherein said pipette and said conduit are arranged relative to one another such that said pipette is inserted into one of the containers into which liquid diluent has previously been dispensed while said conduit is inserted into an empty one of the containers prior to insertion of the pipette therein.

19. The combination set forth in claim 17 wherein said valve means comprises a rotary valve having a metering passage therein, a first conduit for supplying liquid diluent to said metering passage and a second conduit for connecting said passage with said filling conduit, and means for rotating said valve in synchronism with the relative vertical reciprocating movement between said filling conduit and the associated container such that said metering passage and first conduit register for filling the metering passage with said liquid diluent during the time when the filling conduit is removed from said container and said metering passage and said second conduit register during the time when said filling conduit is inserted into the container.

20. The combination set forth in claim 19 wherein said valve rotating means comprising an operating lever secured to said rotary valve and operatively connected to said machine for pivotal movement in response to said relative vertical reciprocating movement between said filling conduit and the associated container to produce said synchronous rotation of said valve.

21. The combination set forth in claim 17 wherein said diluent supply and pump means are actuated by the vertical reciprocating relative movement between said containers and said pipette and conduit.

22. In a serial dilution machine having a diluting pipette supported for movement successively into and out of each container in a row thereof and operable to transfer a measured quantity of liquid from one of said containers to the next, the combination therewith of a filling head attachment comprising a valve block attached to the machine for movement with the pipette, a filling conduit extending from said block for insertion into the container preceding the container registering with the pipette when the pipette is inserted into the latter container, a valve movable in said block for dispensing a measured quantity of diluent via said filling conduit into the associated container and a valve operating link connected to said valve and having an operative connection with a portion of said machine which remains fixed in position during movement of said block such that said block movement causes said link to move said valve to the dispensing position thereof when said filling conduit is inserted into the associated container.

References Cited by the Examiner

UNITED STATES PATENTS
2,624,656 1/53 Andrews et al. 23—259
2,894,542 7/59 Alm 141—130
3,012,863 12/61 Feichtmeir 23—253

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UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,188,181        June 8, 1965

William A. Peterson et al.

It is hereby certified that error appears in the above numbered patent requiring correction and that the said Letters Patent should read as corrected below.

Column 10, line 23, before "interconnected" insert -- operably --.

Signed and sealed this 7th day of December 1965.

(SEAL)
Attest:

ERNEST W. SWIDER
Attesting Officer

EDWARD J. BRENNER
Commissioner of Patents