United States Patent [19]

McKie, Jr.

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[54] **MULTIPLE SOLUTION TESTING DEVICE**

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[73] Assignee: **Pfizer Inc.**, New York, N.Y.

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[21] Appl. No.: **279,069**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 159,795, July 6, 1971, abandoned.

[52] **U.S. Cl.** .... **23/230 R,** 23/230 B, 23/253 R

[51] **Int. Cl.** **G01n 31/20**

[58] **Field of Search** .... 23/230 B, 230 R, 253 R; 424/11, 12

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Primary Examiner—Robert M. Reese

Attorney, Agent, or Firm—Connolly and Hutz

[57] **ABSTRACT**

Drops of different solutions suspended from the perforations of an upper plate are moved down into contact with a suitable reactive layer or substrate disposed on a lower plate, or the drops may bulge above a welled lower plate which is contacted with an upper reactive layer. The reactions between each of the solutions and reactive layer simultaneously commence when the suspended or upwardly bulging drops contact the reactive layer. A sheet of unexposed but developed photographic film incorporating at least one gelatin layer provides a common reactive layer for comparing a number of trypsin-catalyzed reactions. The film is conveniently handled and inspected when attached to an indexed glass plate. The photographic film is immersed in a low pH bath to simultaneously terminate and preserve a record of all of the inherently concurrent reactions. A simple press with a removable cavitied plate and reactive layer advantageously performs the contact.

26 Claims, 16 Drawing Figures

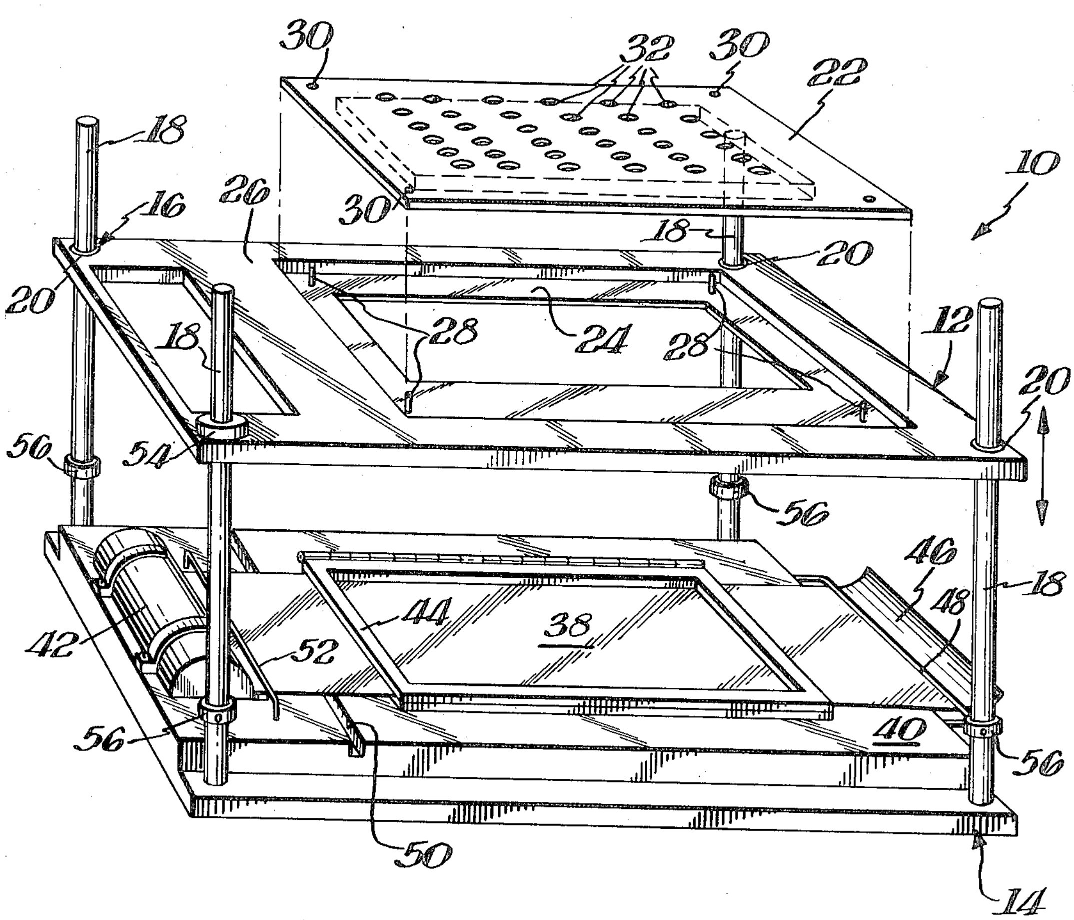

Fig. 1.

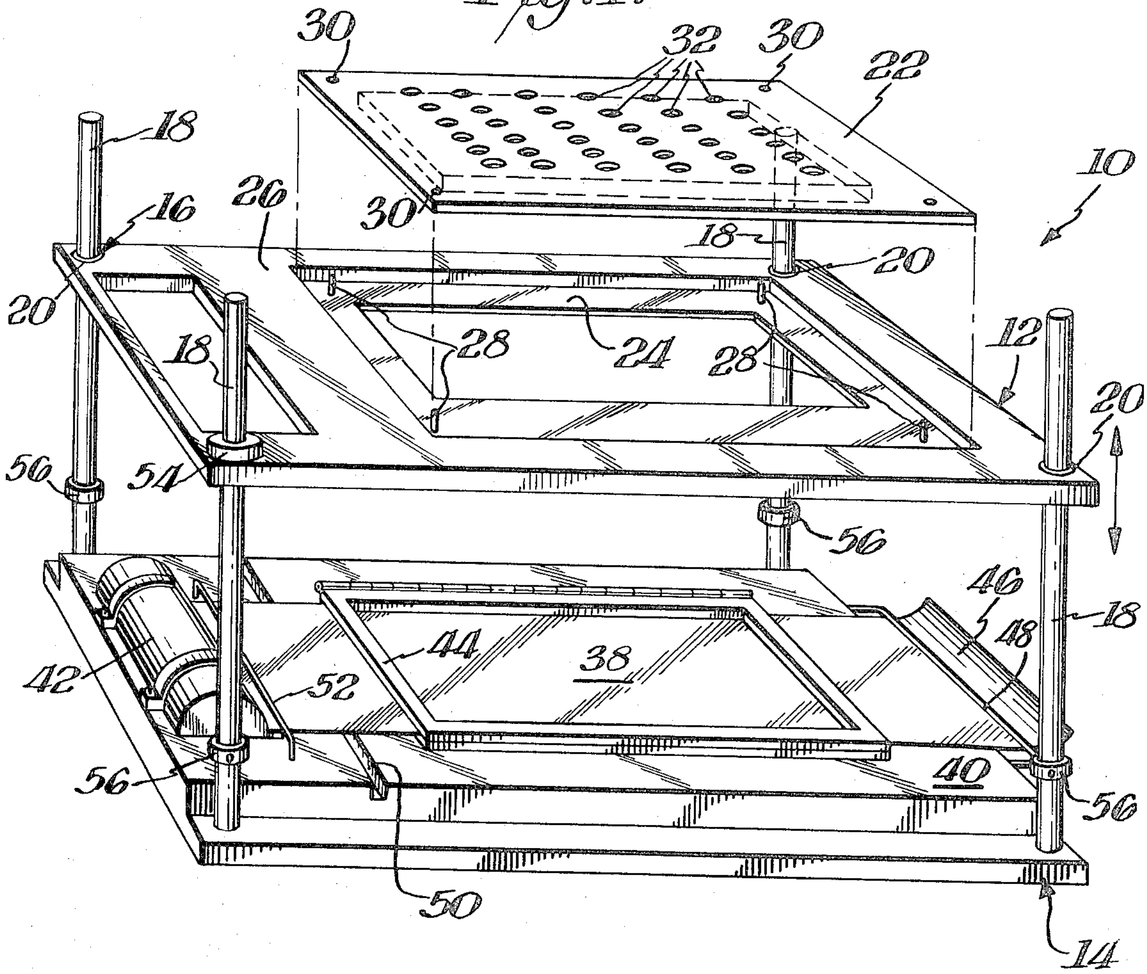
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Fig. 2.

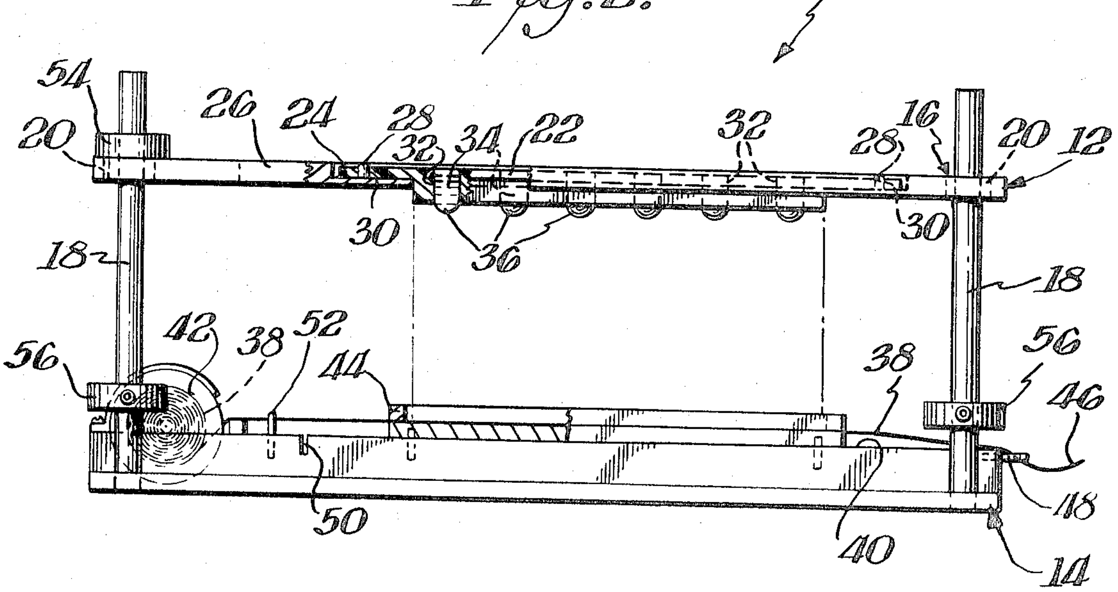
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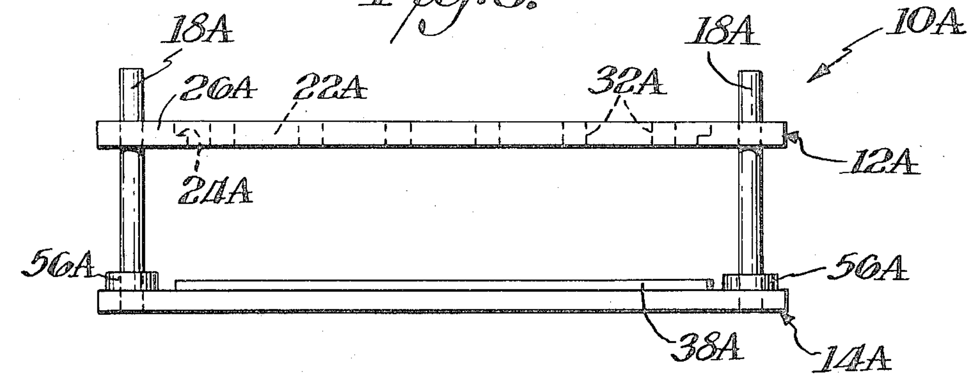
Fig. 3.



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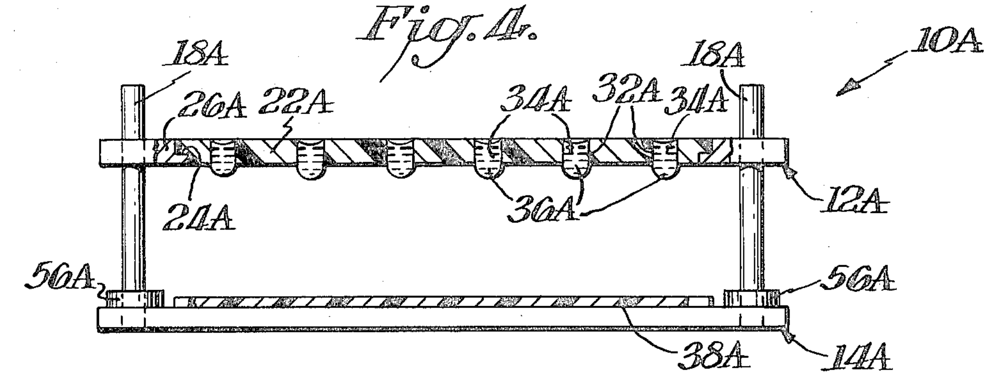


Fig. 5.

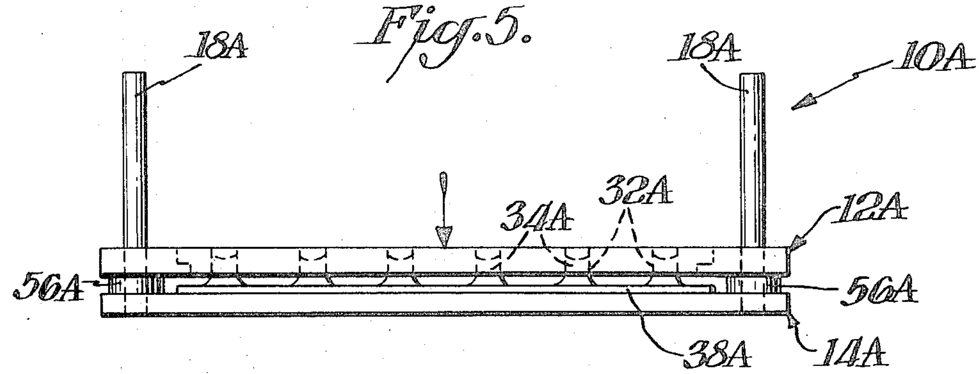
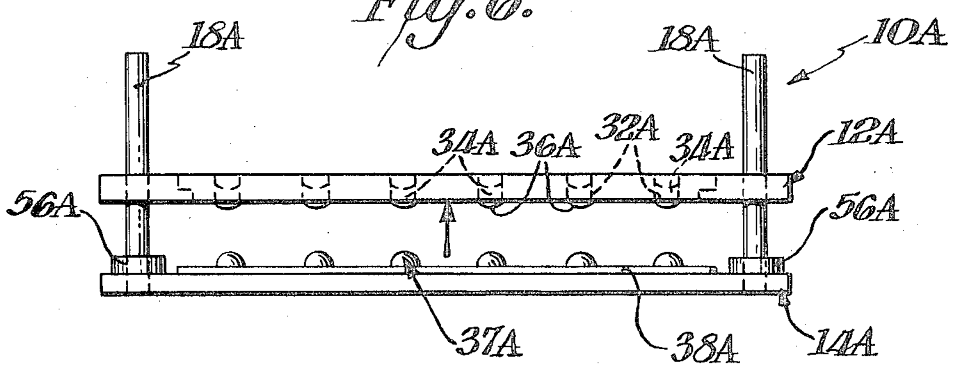


Fig. 6.



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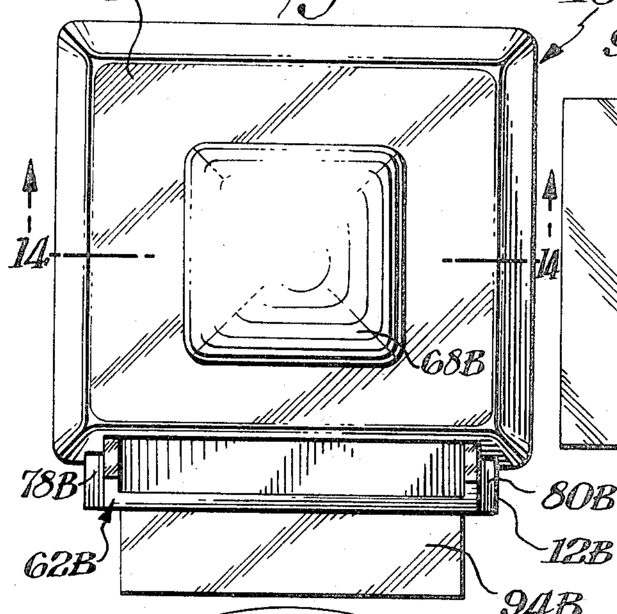


Fig. 12.

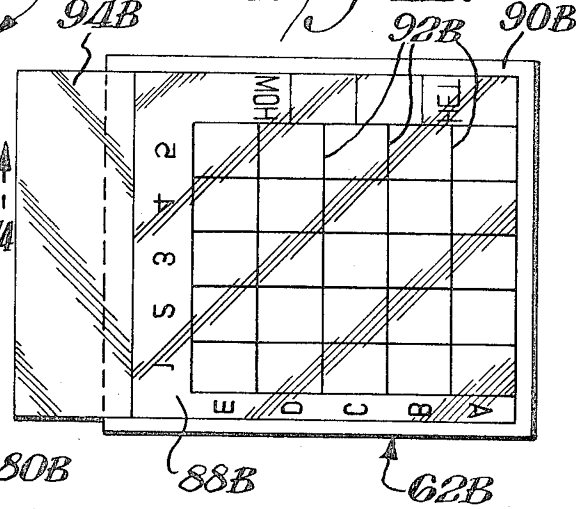
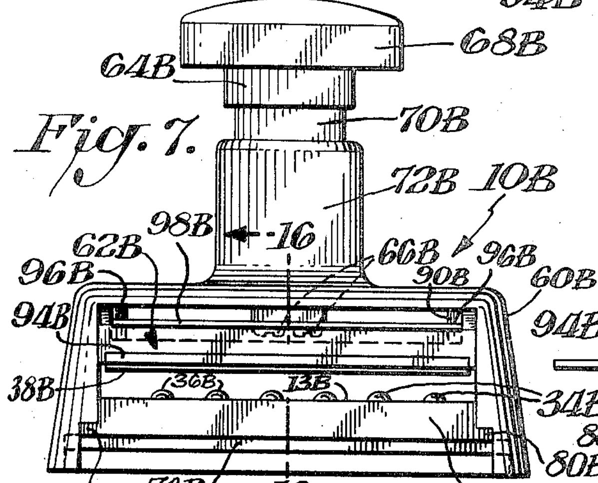


Fig. 7.



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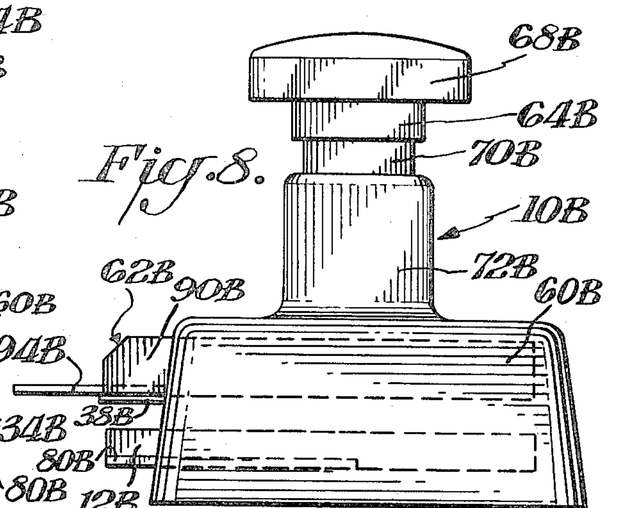


Fig. 10.

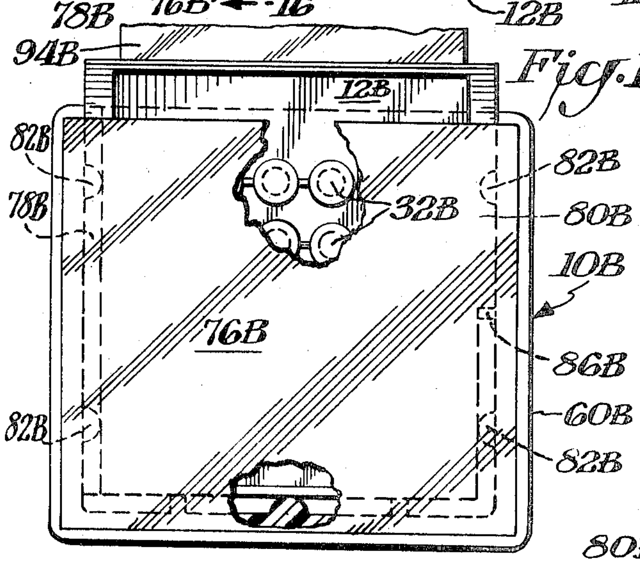


Fig. 11.

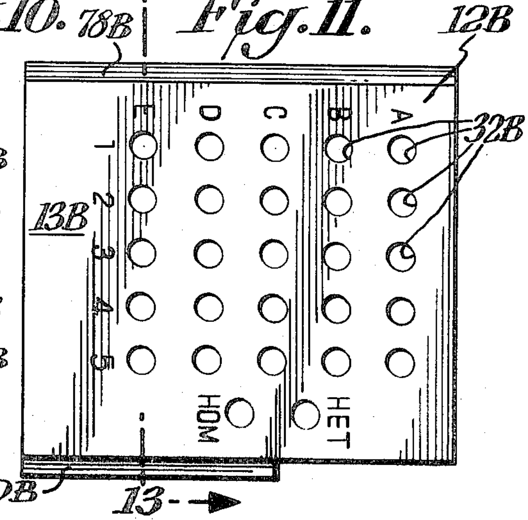


Fig. 13.

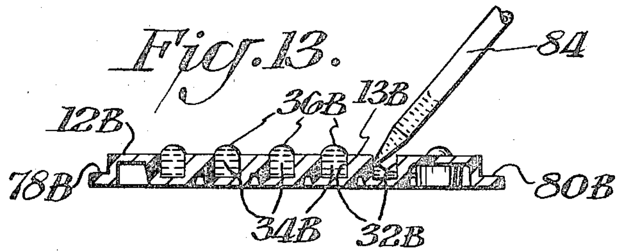


Fig. 14.

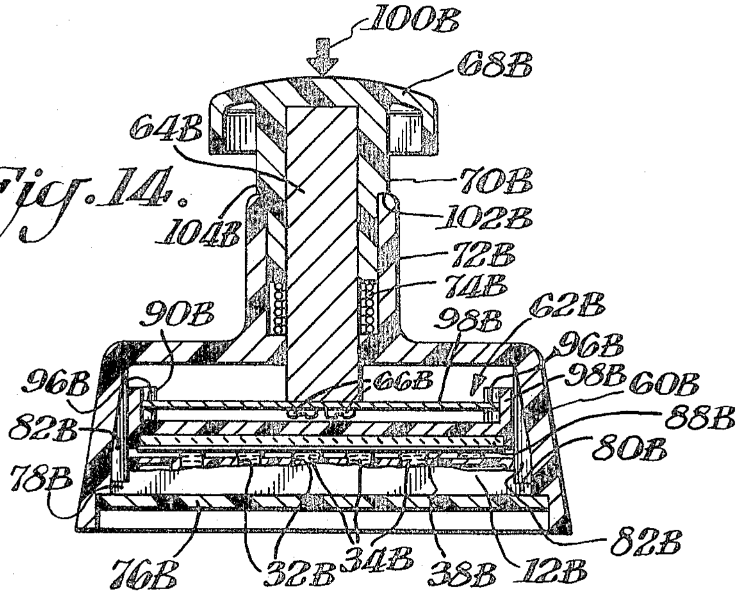


Fig. 15.

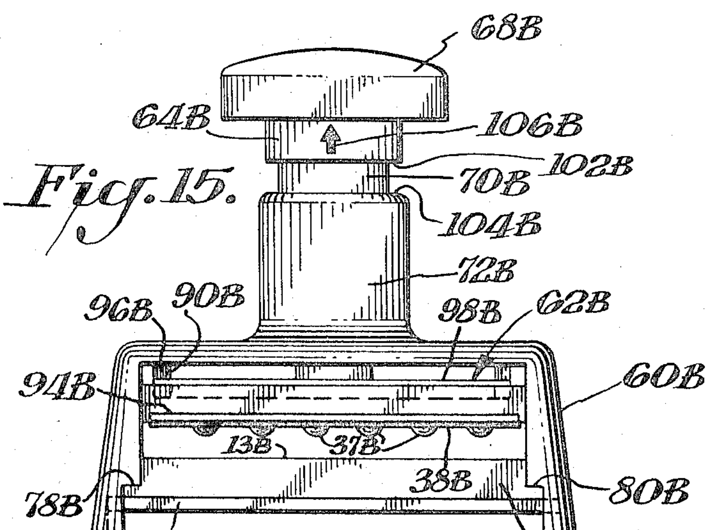
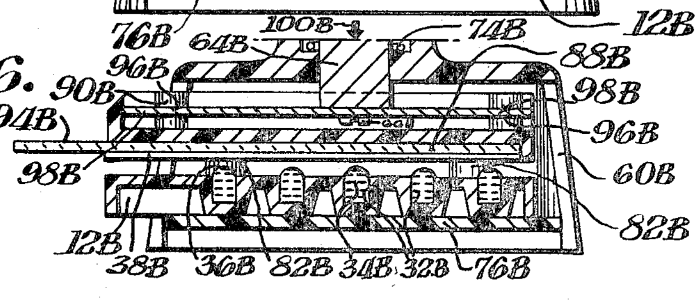


Fig. 16.



MULTIPLE SOLUTION TESTING DEVICE

CROSS-REFERENCES TO RELATED APPLICATIONS

This application is a continuation-in-part of copending application Ser. No. 159,795, filed July 6, 1971, now abandoned.

BACKGROUND OF THE INVENTION

This invention relates to a method and device for simultaneously testing a number of different solutions in a predetermined period of time. Separate timed tests for each solution require painstaking care, considerable laboratory time and still incur the risk of human error. An object of this invention is to provide a simple and economical method and apparatus for testing a number of solutions. Another object is to provide such a method and apparatus for tests which must be of uniform duration.

SUMMARY OF THE INVENTION

In accordance with this invention, a plate having cavities such as a perforated upper plate carrying drops of solution suspended from its holes is moved closely adjacent to a lower plate carrying a reactive substrate. The plate with cavities may also be a lower plate with a number of wells in an upper surface into which an excess of solution is deposited to make the drops bulge up above it. The welled plate is made of a material which is not wet by the solution (hydrophobic) to prevent the drops from spreading out of the wells. An upper reactive layer is moved down into contact with the bulging drops on the welled plate. This simultaneously contacts the drops with the reactive layer and partially transfers an equal volume of each to the reactive layer when the plates are separated. All of the test reactions thus simultaneously commence and can be simultaneously terminated by application of a suitable inhibitor. The upper and lower plates may be movably connected by rods and slide bearings or by a single plunger. Film storage and a holding frame may be conveniently mounted on the lower plate below a perforated upper plate. The welled lower plate may be removably inserted below a reactively surfaced plate which is removably attached to a plunger.

BRIEF DESCRIPTION OF THE DRAWINGS

Novel features and advantages of the present invention will become apparent to one skilled in the art from a reading of the following description in conjunction with the accompanying drawings wherein similar reference characters refer to similar parts in which:

FIG. **1** is partially exploded pictorial view of one embodiment of this invention;

FIG. **2** is a front view in elevation of the embodiment shown in FIG. **1**;

FIGS. **3–6** are schematic side views in elevation of a simplified form of the device shown in FIGS. **1** and **2** in successive phases of operation;

FIG. **7** is a front view in elevation of a further embodiment of this invention in a preliminary phase of operation;

FIG. **8** is a side view in elevation of the embodiment shown in FIG. **7**;

FIG. **9** is a top plan view in elevation of the embodiment shown in FIGS. **7** and **8**;

FIG. **10** is a bottom plan view in elevation of the embodiment shown in FIGS. **7–9**;

FIG. **11** is a top plan view of the drop-holding plate utilized in the embodiment shown in FIGS. **7–10**;

FIG. **12** is a bottom plan view of the composite platen utilized in the embodiment shown in FIGS. **7–10**;

FIG. **13** is a cross-sectional view taken through FIG. **11** along the line **13—13**, and in the process of being filled with drops of solution;

FIG. **14** is a cross-sectional view taken through FIG. **9** along the line **14—14**, but in the downwardly compressed position;

FIG. **15** is a front view in elevation similar to FIG. **7** but in the upwardly returned position subsequent to that shown in FIG. **14**; and

FIG. **16** is a cross-sectional view taken through FIG. **7** along the line **16—16** but in a slightly downwardly compressed position before contact between the lower surface of the platen and the drops held in the lower plate.

DESCRIPTION OF THE PREFERRED EMBODIMENTS (FIGS. 1-3)

In FIG. **1** is shown an apparatus **10** for simultaneously testing the reaction of a number of different solutions with a common reactive substrate. Apparatus **10** includes an upper plate **12** movably mounted relative to lower base plate **14** by coupling **16** including four rods **18** sliding within collar bearings **20.** Upper plate **12** includes a perforated holder **22** removably mounted within recess **24** in frame plate **26.** Positioning pins **28** extend through corresponding holes **30** in perforated holder **22.** Perforated holder **22** includes a number of right cylindrical wells **32.**

The shape of holder **22,** its thickness and material are designed to have an adhesive force for drops **34** of liquid solution to hold a sufficient volume of liquid within and a sufficient distance below wells **32** to contact lower reactive substrate **38,** as later described in detail. Upper holder **22** has an array of holes or wells **32** machined through it (for example right cylindrical) and is made of a material which has lower adhesive forces for some liquid than does the material of lower reactive substrate **38** for the same liquid. If the liquid is water, for example, upper plate **12** could be composed of a plastic and lower reactive substrate of a gelatin film. Other useful shapes for wells **32** are, for example, right elliptical or other right free-form shapes.

A certain volume of liquid is deposited (using a pipette or other convenient volume delivery system) into each well **32** of upper plate **12** forming the suspended drop configuration **36** shown in FIG. **2.** The volume of the liquid required to form a stable (with respect to mechanical disturbance) suspended drop primarily depends upon the density of the liquid, its surface tension, the magnitude of the adhesive forces between the liquid and the surface material of the plate per unit of contact area, and the size and shape of the hole. For example, an extremely stable drop is formed when 50×10^{-6} liters of water are deposited into right cylindrical holes of 3.5 millimeter diameter in a 4 millimeter thick acrylic resin plate as shown in the drawings.

Lower base plate **14** includes upper surface **40** upon which common reactive substrate **38** is disposed for contact by suspended portions **36** of drops **34.** Substrate **38** is, for example, the gelatin surface of an unexposed but developed sheet of photographic film, which is useful in the study of trypsin-catalyzed reactions in which the gelatin substrate emulsion, is hydrolyzed by different concentrations of aqueous trypsin solutions, to form peptide products.

Film **38** is conveniently stored within container **42** mounted at one side of base plate **14** and is maintained in a flat position within hinged framed holder **44.** End **46** of film **38** is conveniently retained within rectangular wire loop **48** at the end of base plate **14** remote from film container **42.** Notch **50** in upper surface **40** of base plate **14,** between film container **42** and holder frame **44,** provides a convenient means for severing used pieces of film **38** from container **42.** Guide wire **52** aligns passage of film **38** through holder frame **44.**

Frictional disc brake **54** attached to one corner of upper plate **12** and in frictional contact with adjacent rod **18** gently holds upper plate **12** in position, and its holding force is easily manually overcome when upper plate **12** is moved throughout its range of operative positions, including the lower position against adjustable stop collars **56.** Frictional disc brake **54** is made, for example, of suitable frictional material such as a resilient compressible washer made of felt or sponge material (synthetic sponge rubber or polyurethane sponge).

Apparatus **10** is useful for conducting a number of simultaneous tests of unknown solutions. It is particularly useful where the reactions of a number of unknown solutions must all be compared with the reaction of standard solutions with the same reactive substrate in a uniform period of time. This device as previously described is particularly useful in determining the trypsin inhibiting capacity of human sera by comparing them side by side with simulated normal, heterozygous and homozygous antitrypsin sera.

Such tests are described in detail in U.S. Pat. No. 3,730,843. Performance of the test described in the aforementioned copending application by conventional methods would require considerable time and care. The method and apparatus of this invention permits a great number of such tests to be simultaneously and accurately conducted.

OPERATION (FIGS. **3–6**)

FIGS. **3–6** illustrate the performance of the method of this invention by a simplified apparatus **10A** shown in FIG. **3** ready for use. A number of trypsin solutions of 50 × 10^{-6} liters are deposited with an automatic pipette into holes or wells **32A** of upper plate **12A** shown in FIG. **4.** Upper plate or carriage **12A** is lowered manually until contact with stops **56A** is made as shown in FIG. **5.**

When contact is made, the liquid samples simultaneously make contact with film **38A** as shown in FIG. **5.** The distance between plates **12A** and **14A** at contact, governed by the size or position of stops **56A,** is one of the determining factors of the magnitude of the contact area of the liquid with film **38A** on lower plate **14A.** At contact, a timer (not shown) is activated either manually or by electrical contact between upper plate **12A** and stop collar(s) **56A.**

Following contact upper plate **12A** is returned to its original position thereby, effecting the partial transfer of an equal volume of each liquid sample in drops **37A** to film **38A** as depicted in FIG. **6.** The efficiency of the transfer can be maximized for any given system and configuration if the reactive substrate-liquid forces are greater than the upper plate-liquid forces. When, for example, 50 × 10^{-6} liters of water are deposited in the right cylindrical holes of 3.5 millimeter diameter, approximately 20 percent of the water is transferred from the upper acrylic plate to a gelatin film reactive substrate when the stops **56A** are set so that a 1 millimeter gap exists between the plates at contact. In contrast, if the gelatin film reactive substrate is replaced with polystyrene, only 10 percent of the water in holes **32A** is transferred to the polystyrene surface.

At some designated time ($t = x$) lower plate or carriage **14A** containing film **38A** and its array of about 10 × 10^{-6} liter drops **37A** of reaction mixture is transferred to a low pH (2–4 pH) bath (not shown). Upon immersion of film **38A**, the catalytic action of the trypsin is terminated and the hydrolyzed gelatin is removed. The extent of hydrolysis of the gelatin is then assessed by transmission of light through the spotted areas of film **38A**. The use of a color film **38A**, as described in the aforementioned copending patent application, provides a tricolored gelatin sandwich in which the depth of reaction penetrating is read with remarkable facility.

(FIGS. 7–16)

In FIGS. 7–16 is shown another testing apparatus **10B** which primarily differs from apparatus **10** and **10A** in that solution drops **34B** are contained within bottomed wells **32B** instead of open holes or wells **32** and **32A.** Plate **12B** further differs in that it is the lower plate from which projecting portions **36B** of drops **34B** project upwardly above plate **12B** instead of being suspended below as shown in FIGS. **1–6.** There are also other corresponding differences as described in the following.

In FIGS. 7 and 8 drop-holding plate or tray **12B** is inserted within chamber **60B** of press apparatus **10B** having a composite platen **62B** secured to plunger **64B** by screws **66B.** Cap **68B** is mounted over and secured to the top of plunger **64B** and its includes a tubular skirt **70B** inserted within sleeve bearing **72B** at the top of press housing **60B.** Compression spring **74B** reacts between the bottom of tubular skirt **70B** and the top of housing **60B** to cause cap **68B** and plunger **64B** to return upwardly after a downward stroke. Removable plate **12B** or tray is inserted above base **76B** of housing **60B** with its flanges **78B** and **80B** inserted under the bottom edges of retaining pins **82B** disposed within housing **60B** in a tongue and groove-like manner.

FIGS. **11, 12** and **13** show details of removable welled plate **12B** including a number of drop-holding bottomed wells **32B** holding drops **34B** of solution. FIG. **13** shows wells **32B** being filled with predetermined excess amounts of solution from pipette **84** sufficient to cause upper projecting portions **36B** of solution to project above the upper surface **13B** of plate **12B** to cause contact with composite platen **62B** and the reaction layer **38B** on it later described. The material of which plate **12B** is made of a type which will not be wet by the contained solution (hydrophobic) therefore preventing the solution from spreading over the surface of the plate and causing it to form a convex meniscus comprising projecting portion **36B** shown in FIG. **13.** The result achieved is that for each liquid sample placed in a well there occurs a convex meniscus of the liquid of a given height above the plate surface. For example, when each 3/16 inch diameter, 3/16 inch deep well of the present injection-molded polystyrene plate is filled with 0.10 milliliters of liquid sample, there results a convex meniscus the maximum height of which is 1.2 mm above the upper surface of the plate. The reason for the preference of this type of plate as opposed to the plate containing the perforated holes from which the liquid samples are suspended is one of convenience. It has been found that less skill and time is required to deliver the sample to a well than into a hole and, more importantly, that the well principle eliminates the possibility of the sample falling through the plate due to a too rapid release of the liquid sample from the pipette.

Plate **12**B is for example injection molded of polystyrene to provide wells **32**B in its upper surface and flanges **78**B and **80**B. Flange **78**B as shown in FIG. **11** extends the entire length of plate **12**B and **80**B extends less than the full length to index plate **12**B for insertion in only one position into press housing **60**B in conjunction with stop **86**B (FIG. **10**). FIG. **11** also shows identifying coordinates A–E and **1–5** for wells **32**B and also for the two control drop-containing holes **32**B designated HET and HOM.

FIG. **12** shows the bottom of the removable elements of platen **62**B including a coordinated glass plate **88**B removably adhered, for example by rubber cement, to the bottom of platen frame **90**B. Coordinated glass **88**B includes ruled blocks **92**B and indicia corresponding to positions of wells **32**B in lower plate **12**B. Glass plate **88**B also includes an extending tab **94**B to provide a handle for the removable assembly shown in FIG. **12.** Tab **94**B may accordingly be roughened to facilitate grasping and application of identifying marks.

The transparent film-holding plate **88**B is silk-screen coded on its upper surface (non-film surface). The coding design matches the particular array of samples on the film as dictated by the spacing of wells on the well plate. In the particular case of the alpha-1-antitrypsin mass screening test, this coding facilitates the rapid identification of any positive reaction when light is passed through the normally opaque dye/gelatin film substrate and further facilitates the comparison of a positive sample spot with the two reference spots labeled HET (heterozygote deficiency) and HOM (homozygote deficiency).

FIG. **7** shows assembled frame **90**B and glass plate **88** B inserted in housing **60**B with lateral slots **96**B of frame **90**B engaged about the edges of platen-supporting plate **98**B, which is secured to the bottom of plunger **64**B by screws **66**B. In FIGS. **7, 14–16** is also shown reaction layer **38**B of unexposed but developed color film as previously described. Reaction layer **38**B is adhered to the bottom of glass plate **88**B by a suitable adhesive, such as rubber cement.

OPERATION (FIGS. 7–16)

Drops of different solutions are placed in wells **32**B by pipette **84** as shown in FIG. **13** to cause an excess of solution to raise projecting menisci **36**B of solution above upper surface **13**B of plate **12**B. Plate **13**B is then inserted within the housing **60**B of base **76**B as shown in FIGS. **7–10.** A piece of unexposed but developed color film **38**B corresponding in area to the coordinated portion of glass plate **88**B is then attached to the bottom of glass plate **88**B by a liquid adhesive, such as rubber cement or for example a double-sided adhesive transparent tape. Reaction layer or film **38**B is mounted or bonded to glass plate **88**B on the unreactive side of the film, that is by its celluloid or other backing which is bound to the glass by means of an adhesive.

A composite platen **62**B is then prepared as shown in FIG. **12** by adhering glass plate **88**B to the bottom of frame **90**B which is then removably connected to plunger **64**B by engaging grooves **96**B about platen supporting plate **98**B. Platen **62**B is then moved toward projecting drop menisci **36**B, as shown in FIG. **16** and ultimately in FIG. **14** to cause reactive layer **38**B to contact projecting menisci **36**B of drops **34**B. As a result of downward pressure in the direction of arrow **100**B on plunger cap **68**B, the downward motion of plunger **64**B is arrested by contact of shoulder stop **102**B of cap skirt **70**B with the upper periphery **104**B of housing sleeve bearing **72**B. Stop surfaces **102**B and **104**B are predetermined to cause menisci **36**B to simultaneously touch the surface of film **38**B a predetermined amount sufficient to cause the transfer of drop portions **37**B shown in FIG. **15** of sufficient volume upon upward movement of plunger **64**B in the direction of arrow **106**B to initiate substantial reactions for the particular comparison test being conducted. Return movement of plunger **64**B is accomplished by releasing cap **68**B which is automatically raised by return expansion of compression spring **74**B. The size of the transferred drops **37**B may be adjusted by changing the dimensions of surface **102**B and **104**B by adjusting configurations or inserting stop washers (not shown).

Film **38**B containing transferred drops **37**B may be left in position in housing **60**B until the reaction time is completed or may be removed together with glass plate **88**B and frame **90**B (shown in FIG. **12**) and stored until the end of the reaction time. Since film **38**B is hydrophilic it can be stored upright or on its side without any danger of drops **37**B running together. After the reaction time is completed and film **38**B immersed to terminate the reaction, the extent of hydrolysis of the gelatin for each sample may be read from the coordinated assembly shown in FIG. **12** together with the adhered film layer **38**B. Drop-containing plate **12**B is removed from housing **60**B and discarded after transfer of projecting menisci **36**B of drops **34**B.

As one skilled in the art can readily appreciate, the plate **88**B can be composed of a wide variety of materials other than glass, including plastics, metals or wood. Further, plate **88**B can be solid or may comprise a rigid frame to which the film **38**B is adhered.

I claim:

1. A method of simultaneously testing the reactions of a number of solutions with a reactive layer which comprises the steps of placing substantially equal quantities of each of said solutions in cavities in a holder whereby substantially equal quantities of said solutions are caused to project substantially equal distances from said holder, disposing said reactive layer upon a plate, aligning said holder and said plate parallel to each other whereby each of the projecting quantities is equidistant from said reactive layer, causing relative movement between said holder and said plate while maintaining said parallel relationship for disposing them closely adjacent each other whereby said projecting quantities simultaneously contact said reactive layer to simultaneously commence reactions between said solutions and said reactive layer, and separating said holder from said plate whereby substantially equal portions of said projecting quantities transfer to said reactive layer for completing said reactions.

2. A method as set forth in claim 1 wherein an inhibitor is applied to said transferred quantities to simultaneously terminate said reactions whereby said reactions are made substantially concurrent with each other.

3. A method as set forth in claim 1 wherein said quantities include standard solutions to which the other reactions are compared and evaluated.

4. A method as set forth in claim 1 wherein a record of said reactions is preserved upon said reactive substrate.

5. A method as set forth in claim 1 wherein said portions of said quantities are caused to project below said holder by suspension of said portions below said holder.

6. A method as set forth in claim 1 wherein said portions of said quantities are caused to project from said holder by causing a meniscus to project above said holder.

7. An apparatus for simultaneously testing the reaction of a number of solutions with a reactive layer comprising a solution-holding plate having a number of equal cavities which are constructed and arranged for independently holding and supporting substantially equal quantities of said solutions projecting substantially equal distances from said solution-holding plate, a reaction plate having portions disposed parallel to and equidistantly from corresponding portions of said solution-holding plate, said reaction plate having supporting means for holding said reactive layer, parallel-acting movable coupling means connecting said solution-holding and reaction plates whereby the relative distances between said parallel plates may be parallelly varied from substantially separated to closely adjacent positions, said cavities having a shape and size for retaining said quantities of solution with a portion thereof projecting at said substantially equal distances therefrom whereby said projecting portions simultaneously contact said reactive layer on said reaction plate when said plates are moved into said closely adjacent positions and for substantially transferring equal quantities of said solutions to said reactive layer on said reaction plate upon subsequent separation of said plates.

8. An apparatus as set forth in claim 7 wherein said cavities comprise holes through said plate, said projecting portions of said quantities being suspended from said holes below said plate, and said reaction plate being disposed below said solution holding plate.

9. An apparatus as set forth in claim 7 wherein said movable coupling means includes a holding element whereby said solution holding plate is maintained in a separated position from said reaction plate.

10. An apparatus as set forth in claim 7 wherein said movable coupling means includes adjustable stops for varying the distance between said projecting quantities and said reaction plate in said closely adjacent position of said plates.

11. An apparatus as set forth in claim 7 wherein said cavities are substantially cylindrical.

12. An apparatus as set forth in claim 11 wherein said cavities have a width of several millimeters.

13. An apparatus as set forth in claim 7 wherein said movable coupling means comprises rod and slide bearing means.

14. An apparatus as set forth in claim 13 wherein said solution-holding plate comprises a frame plate and a perforated insert plate, said perforated insert plate being removably mounted in said frame plate.

15. An apparatus as set forth in claim 7 wherein a removable sheet is disposed on said reaction plate for contact with said drops, and a framed holder being mounted upon said reaction plate for receiving and holding said sheet flat upon said reaction plate.

16. An apparatus as set forth in claim 15 wherein said sheet comprises photographic film, and a film container facing mounted upon said reaction plate for supplying said film to said holder.

17. An apparatus as set forth in claim 7 wherein said cavities comprise wells in said plate, said projecting portions of said quantities being menisci of said drops projecting above said drop-holding plate, and said reaction plate being disposed above said solution holding plate.

18. An apparatus as set forth in claim 17 wherein said solution holding plate comprises a shallow tray incorporating a number of said wells.

19. An apparatus as set forth in claim 18 wherein said tray and said movable coupling means include tongue and groove means for removable engagement with each other.

20. An apparatus as set forth in claim 17 wherein said movable coupling means comprises a press having a base and a movable plunger disposed above said base, said base having receiving means for insertion of said solution holding plate, said plunger having removable attaching means for engagement by said reaction plate, and stop means reacting between said plunger and said base for terminating the movement of said plunger toward said reaction plate.

21. An apparatus as set forth in claim 20 wherein said layer has a platen-supporting plate attached to said plunger, a platen removably engaged with said platen-supporting plate, said platen including a frame, and detachable means removably connecting said platen with said frame.

22. An apparatus as set forth in claim 21 wherein said detachable means comprises slots in said frame and engaging edges of said platen-supporting plate.

23. An apparatus as set forth in claim 21 wherein said platen and said solution plate have portions projecting a short distance outwardly from said base to facilitate insertion and removal of said platen and said solution holding plate.

24. An apparatus as set forth in claim 21 in which a transparent coordinate plate is attached to the lower surface of said frame.

25. An apparatus as set forth in claim 24 wherein said transparent plate comprises a glass plate.

26. An apparatus as set forth in claim 24 wherein a slot is disposed on said reaction plate between said film container and said film holder to facilitate the severing of portions of said film in said holder from said container.

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