

Dec. 9, 1969

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3,482,943

REAGENT DEPOSITION DEVICE

Filed Feb. 14, 1966

2 Sheets-Sheet 1

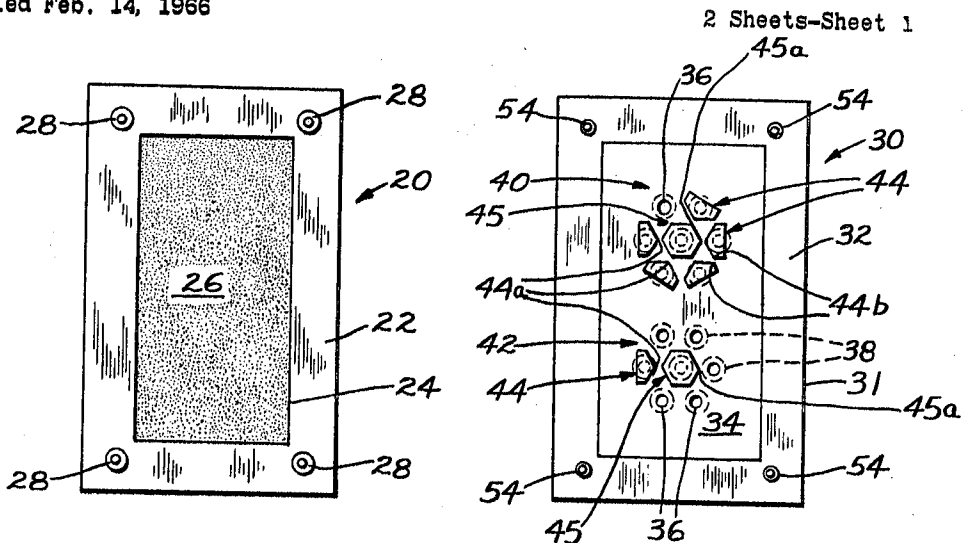


FIGURE 1.

FIGURE 2.

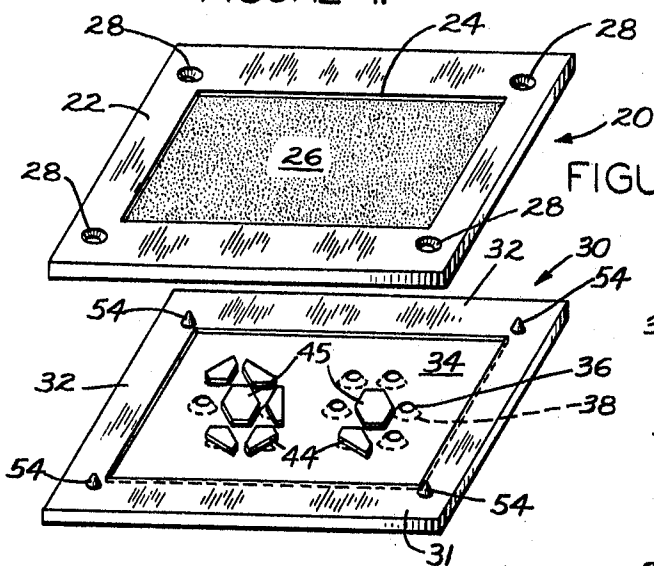


FIGURE 3.

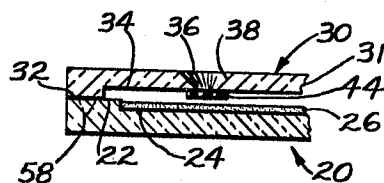


FIGURE 6.

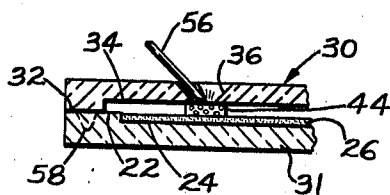


FIGURE 7.

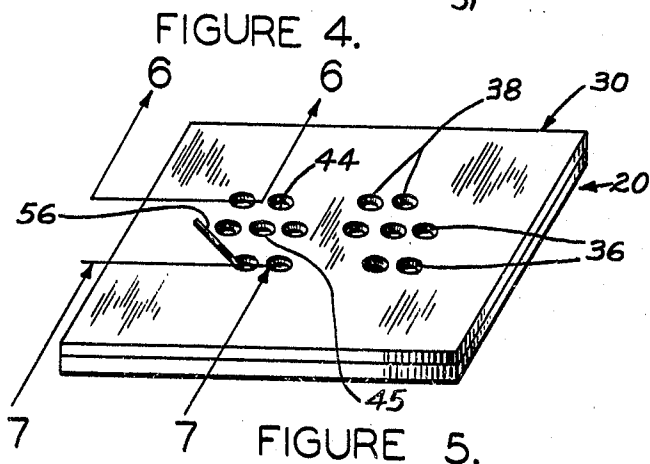


FIGURE 5.

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2 Sheets-Sheet 2

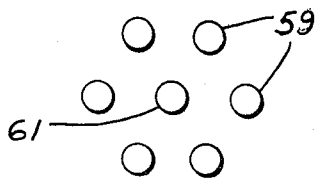


FIGURE 8.

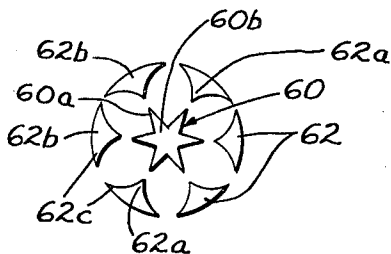


FIGURE 9.

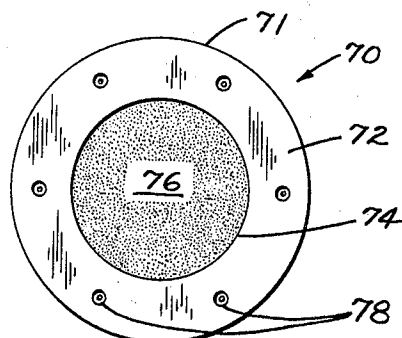


FIGURE 10.

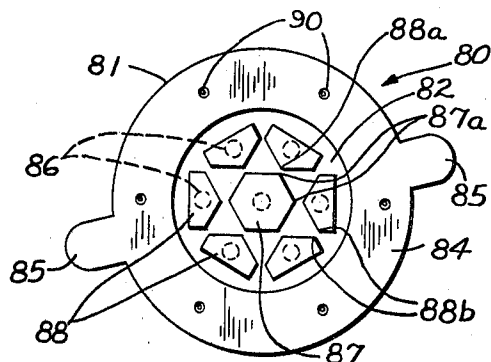


FIGURE 11.

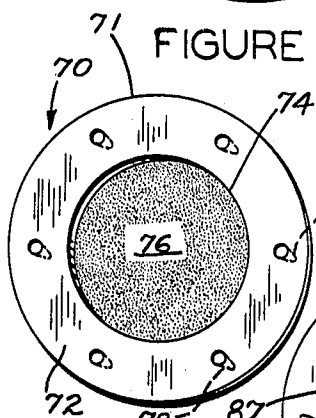


FIGURE 12.

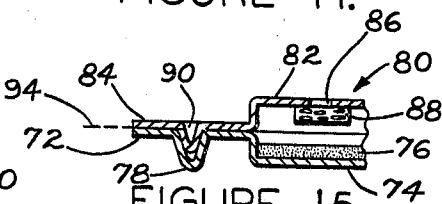


FIGURE 13.

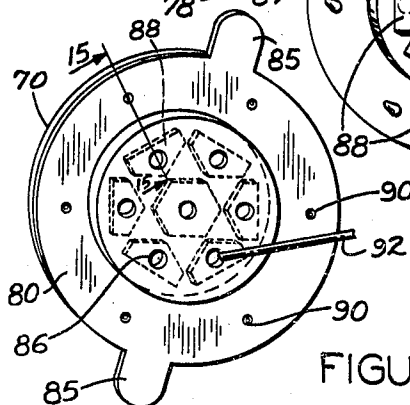


FIGURE 14.

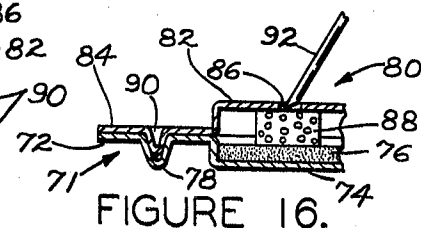


FIGURE 15.

FIGURE 16.

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3,482,943

REAGENT DEPOSITION DEVICE

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U.S. Cl. 23—253

15 Claims

ABSTRACT OF THE DISCLOSURE

A device for depositing reagents including a bibulous material secured to a carrier member and a receiving surface spaced from the bibulous material particularly useful in conducting immunodiffusion tests.

This invention relates to a deposition device for reagent-containing liquids. More particularly it enables reagent-containing liquids to be deposited on receiving surfaces such as gel layers for conducting biological and precipitation tests.

The prior art deposition methods include (1) forming in a gel layer wells which can be used to hold reagent-containing liquids, and (2) absorbing the liquid into paper discs and placement of the discs onto the surface of a gel layer. Funnel devices have also been used to channel liquids onto gel surfaces.

Difficulties are inherent in these prior practices. Wells in thin gel layers must be precision cut because of the danger of mechanically rupturing the layer. Liquids must be carefully added in order to avoid spilling of reagent or overflow from the well. When paper discs are used they must be accurately placed in a wet state onto the gel layer and care must be taken to avoid dropping some of the liquid on the layer, mechanically rupturing the surface of the gel or separating the gel from its support. If the discs contain too small a quantity of liquid the testing may be impaired, whereas the use of funnels tends to allow flooding and tearing of the gel surface which ruins the test.

It is therefore an object of this invention to provide a reagent deposition device which allows positioning of a bibulous material deposition member over a receiving surface in a dry state and subsequent depositing of a liquid through the bibulous member without the possibility of flooding.

Another object is to provide a reagent deposition device of the class described wherein the bibulous deposition member is fluid expandable and is initially positioned in a dry state out of contact with a gel layer, said member being expandable into contact with the gel layer by impregnation with a liquid containing the reagent.

Yet another object is to provide a reagent deposition device for carrying out micro-Ouchterlony immunoprecipitin tests with improved sensitivity and facility.

Another object is to provide a reagent deposition device which has associated therewith spacing means and registration means for positioning a bibulous deposition member with respect to a receiving surface.

Yet another object is to provide a reagent deposition device which employs a plurality of bibulous material deposition members and thereby allows simultaneously testing with several reagents.

The device of this invention has a wide range of utility. It can be used to deposit single reagents or a multiplicity of reagents onto the surfaces of various testing media. Primary among these media are gel layers on which various biological and precipitation tests are conducted. The device may also be used to deposit reagents pursuant to immunoelectro-osmosis and electrophoretic studies. Disc

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test wherein various materials are deposited on pre-inoculated gel layers to accelerate or retard bacterial growth on the gel may be advantageously conducted by employing the device of the present invention. Another main utility is the depositing of a reagent which precipitates with another reagent in the testing media.

The primary employment of a testing procedure based on such precipitation is in an immunological test which employs a gel layer and is known as the Ouchterlony immunodiffusion precipitin method. In this method a quantity of an antigen is introduced into a gel layer at a first position and a suspected antibody-containing sample is introduced at a second position which is spaced from the first position. If a line of precipitation forms in the gel between the two positions the conclusion can be made that the sample introduced contained antibody for the particular antigen. By studying relative positioning and density of the precipitation lines the antibody concentration or the antigen concentration can be estimated.

By using the device of this invention to introduce immunological reagents onto gel surfaces for diffusion into the gel layers, the absence, presence, increase or decrease of immunological reagents in various fluids may be detected. For blood plasma components one can establish such information for gamma-globulin, genetic globulin, haptoglobin, fibrinogen, B₂ macroglobulin (Waldenströms), C-reactive protein and myeloma protein. For forensic medicine determinations it is also possible to identify the species source of a given blood sample. Information concerning Bence-Jones protein in urine can be elicited by use of the present device in a more advantageous manner than by following the prior art.

Various antibodies and antigens in body fluids such as blood, urine, spinal fluids, cerebrospinal fluid, pleural fluid, peritoneal fluid, and synovial fluids may be detected and quantified by employing the device of the present invention for reagent deposition onto gel layers. Bacterial diseases such as tuberculosis, cholera and brucellosis can be tested for, as can mycological diseases such as histoplasmosis, cryptococcosis, aspergillosis, Listeriosis, and coccidiomycosis. Other uses are detection of parasitological diseases such as amebiasis, malaria, and toxoplasmosis; viral diseases such as pox virus, echo virus, and Coxsackie virus; and allergenic disorders caused by various inhalants such as ragweed.

For detection of many of these diseases the presence of the antibody to the organism or inhalant is tested for in a blood fraction sample by employing a known amount of the organism or inhalant. The procedure can be reversed, of course, to test for the antigenic material.

The antibody and antigen can be simultaneously added at different locations on the gel layer by use of the present device or may be added according to a timed relationship.

These and other features and advantages will appear to those familiar with this art from the following detailed description of two embodiments thereof which appear in the accompanying drawings.

In the drawings:

FIGURE 1 is a plan view of a rectangular dish containing a gel layer;

FIGURE 2 is a plan view of a reagent deposition device of the present invention adapted for use with the dish of FIGURE 1;

FIGURE 3 is a perspective plan view of the dish of FIGURE 1;

FIGURE 4 is a perspective plan view of the deposition device of FIGURE 2;

FIGURE 5 is a perspective plan view of the device of FIGURE 2 inverted and resting in assembled relation upon the dish of FIGURE 1;

FIGURE 6 is an enlarged sectional view taken on line 6—6 of FIGURE 5 showing a bibulous deposition member in a dry state and spaced from the gel layer;

FIGURE 7 is an enlarged sectional view taken on line 7—7 of FIGURE 5 showing a bibulous deposition member expanded into contact with the gel layer following impregnation with a liquid;

FIGURE 8 shows an arrangement of disc-shaped bibulous material deposition members which can be employed in the device of FIGURE 2;

FIGURE 9 shows an arrangement of star and wedge-shaped bibulous material deposition members which can be used in the device of FIGURE 2;

FIGURE 10 is a plan view of a preferred circular modification of the dish of FIGURE 1;

FIGURE 11 is a plan view of a preferred circular modification of the deposition device of FIGURE 2 which is adapted for use with the dish of FIGURE 10;

FIGURE 12 is a perspective plan view of the dish of FIGURE 10;

FIGURE 13 is a perspective plan view of the deposition device of FIGURE 11;

FIGURE 14 is a perspective plan view of the device of FIGURE 13 inverted and resting in assembled relation upon the dish of FIGURE 12;

FIGURE 15 is a sectional view taken on line 15—15 of FIGURE 14 showing a bibulous deposition member in a dry state and spaced from the gel layer; and

FIGURE 16 is a sectional view similar to FIGURE 15 showing the bibulous deposition member expanded into contact with the gel layer after a fluid has been added to said deposition member.

Referring now to FIGURE 1, receiving surface support means 20 is provided in the form of a rectangular dish having flat marginal surface portions 22 and a depressed rectangular central area 24 which contains means 26, such as a gel layer of agar, cellulose acetate or similar material, providing a receiving surface. The dish 20 is preferably provided with conically shaped recesses 28 formed in the surface 22, conveniently near the corners thereof as shown.

A deposition device 30 is illustrated in FIGURE 2 and comprises a rectangular plate-like carrier member 31 having on one side thereof a flat surface portion 32 which extends peripherally around a central rectangular recessed portion 34. A plurality of conical apertures 36 extend through the recessed portion 34 of the carrier member 31 and have their large diameter end portions 38 opening outwardly away from the recess 34. As shown in FIGURE 2 the apertures 36 are arranged in first and second patterns 40 and 42, respectively, in each of which a central aperture is surrounded by a plurality of similar apertures which are spaced equally from the central aperture and are spaced from each other.

A plurality of fluid expandable deposition members 44 and 45 are suitably secured as by gluing to the surface of the recess 34 in covering relation with respect to the inner ends of the respective apertures 36. Deposition members 44 and 45 are preferably made from a compressed, dry cellulose sponge material which when wetted by a liquid tends to return to its normal uncompressed state and expands in the direction of its compression, i.e. thickness. In pattern 40 the central aperture is covered by a hexagonal-shaped member 45 and all but one of the remaining apertures are shown covered by pentagonal-shaped members 44. In pattern 42 the central aperture is covered by a member 45 and only one of the remaining apertures 36 is covered by a member 44. The members 44 may completely cover the associated apertures as shown in FIGURE 2, or may cover only a part of the full area thereof so long as they are aligned in a manner to overlap at least a portion of the associated apertures.

The central bibulous material member 45 preferably is formed with a plurality of peripheral apex portions 45a, and the members 44 preferably each have an apex portion

44a. As shown in FIGURE 2 the members 44 and 45 are arranged with the apex portions 44a and 45a in alignment so that said portions of the members 44 and 45 more closely approach each other than do other more central or main body portions of said members. Preferably each of the members 44 is provided with a pair of additional apex portions 44b so that each member 44 has a pair of apex portions 44b available for alignment with an apex portion 44b of each adjacent member 44.

Carrier member 31 is provided with raised conical nibs 54 integral therewith and projecting from the peripheral surface portion 32 in positions to cooperate with the conical recesses 28 of dish 20 to provide for registration of the carrier member 31 and dish 20 when placed in assembled relation. While nibs 54 and recesses 28 have been illustrated as the registration means, any other suitable means such as clamps, collating means, and jigs can be employed to register carrier member 31 with dish 20.

FIGURES 3 to 7 illustrate the use of deposition device 30 and dish 20. Deposition device 30 of FIGURE 4 is manually inverted and placed onto dish 20 of FIGURE 3 so that conical nibs 54 of device 30 seat within the conical recesses 28 of dish 20. After deposition device 30 and dish 20 have been assembled as shown in FIGURE 5 reagent-containing liquid is dispensed onto the deposition members 44 and 45. This is accomplished with respect to a selected deposition member by inserting a hand-held micropipette 56 into the large diameter outer end portion 38 of the associated aperture 36 and contacting the surface of said deposition member therewith. The stronger capillary action of the bibulous material deposition member draws the reagent-containing liquid from the micropipette since only relatively weak capillary forces are exerted in the latter. The flow of liquid from micropipette 56 is controlled by the capillary action of the contacted deposition member which absorbs only enough liquid to become saturated and thus avoids any danger of overflow of liquid onto the gel layer.

FIGURE 6 shows a sectional view through a portion of the assembled deposition device 30 and dish 20 of FIGURE 5 before a reagent-containing liquid is added to deposition member 44, and it will be observed that the rectangular recessed-portion 34 of device 30 overlies the rectangular recessed central area 24 of dish 20 and the gel layer 26 continued therein. The spacing is such that the lower surface of each deposition member 44 is spaced from the upper surface of gel layer 26, and the same is true of the members 45 prior to wetting thereof.

The expansion of each of said deposition members upon being wetted is sufficient to drive them into intimate contact with the underlying gel layer. Such expansion assures proper contact with the gel layer without requiring precise tolerances of spacing between dish 20 and deposition device 30. FIGURE 7 illustrates the expansion of a deposition member 44 into contact with the gel layer 26 as a result of saturation thereof by a reagent-containing liquid absorbed from a micropipette 56 shown extending into the associated conical aperture 36 and in contact with said deposition member.

It will be appreciated that due to the greater liquid volume in the expanded deposition members a greater quantity of liquid may diffuse into the gel layer than is possible by either the formed well or paper disc techniques of the prior practices.

An example of the use of deposition device 30 and dish 20 is the immunodiffusion testing of blood plasma. For such testing the deposition device 30 is assembled with dish 20 as shown in FIGURE 5, and a separate liquid suspension of each of a plurality of blood plasma antibody components is respectively added by micropipette to each of deposition members 44 arranged in pattern 40. A blood plasma sample is then added to central deposition member 45 by micropipette. The absorption of the liquids by the bibulous deposition members causes them to expand into intimate contact with gel layer 26. The blood plasma

antibody components from members 44 and the blood plasma sample from member 45 then diffuse through gel layer 26 and visible precipitin lines are formed by their interaction when the sample contains antigens corresponding to the individual antibody components. These precipitin lines are formed in gel layer 26 between apex portion 44a of the member or members 44 containing a reacting antibody component and the aligned apex portion 45a and are disposed generally perpendicularly with respect to the alignment of said apex portions.

FIGURES 8 and 9 represent other configurations which may be employed for the bibulous material deposition members 44 and 45 of FIGURE 2. In FIGURE 8 deposition members 59 and 61 in the form of discs are arranged in the same pattern as the deposition members 44 and 45 respectively of FIGURE 2. In FIGURE 9 the centrally disposed deposition member 60 has the form of a six pointed star, and the surrounding members 62 are generally triangular or wedge shaped. Each member 62 has a first apex portion 62a which is directed toward and aligned with a point or apex portion 60a of star 60. Each of the members 62 has a pair of second apex portions 62c which are in general alignment with the nearer apex portions 62c of the next adjacent members 62. The general arrangement of having apex portions or points of each of the bibulous material deposition members spaced closer to each adjacent member than the central or main body portion 62b of each of the members permits close spacing of the members and yet maximizes the area of the gel in which the precipitin lines may form.

FIGURE 10 shows a preferred modification of a receiving surface support means 70 in the form of a circular dish 71 which has a flat marginal portion 72 surrounding a depressed central area 74 which contains means providing a receiving surface such as a gel layer 76. Conical recesses 78 are formed in the peripheral portion 72, preferably equidistant from one another and from the center of dish 71 as shown.

FIGURE 11 shows a preferred modification of a reagent deposition device 80 which comprises a circular plate-like carrier member 81 having a depressed circular central portion 82 surrounded by a flat circular peripheral portion 84. Integral tab portions 85 extend in diametrically opposite directions from the peripheral portion 84 and provide convenient handles for the device 80. The plate 81 may be made of a nonresilient flexible material, and if so, the tabs 85 can, when the device 80 is placed in assembled relation with respect to the dish 71, be folded around the edge of the marginal portion 72 of said dish to thereby also provide means for securing dish 71 to plate 81.

Seven apertures 86 extend through depressed portion 82 of carrier member 81. As shown in FIGURE 11 apertures 86 are arranged in a pattern similar to patterns 40 and 42 of FIGURE 2 in that six apertures are spaced equidistant from a centrally located aperture and from one another. The centrally located aperture is covered by a hexagonal-shaped bibulous expandable deposition member 87 which is similar to the members 45 of FIGURE 2 and is suitably secured to the surface of depressed portion 82. In a like manner pentagonal-shaped, bibulous expandable deposition members 88 similar to the members 44 of FIGURE 2 are suitably secured to the surface of depressed portion 82 and cover the surrounding circularly arranged apertures. Deposition members 87 and 88 are formed with apex portions 87a and 88a aligned with one another in the same manner as the apex portions of the deposition members of FIGURE 2. Also, deposition members 88 have apex portions 88b aligned with respect to like adjacent apex portions on adjacent deposition members 88 in the same manner as members 44 of FIGURE 2.

Carrier member 81 is provided with conical nips 90 integral therewith and projecting from the surface of the peripheral portion 84 in positions to cooperate with conical recesses 78 of dish 71 in providing for coaxial registration of carrier member 81 and dish 71 when placed in assembled relation. The conical recesses 78 of dish 71 are of a

size to snugly receive the conical nips 90 of carrier member 81.

FIGURES 12 to 16 illustrate the use of deposition device 80 and dish 71. The deposition device 80 is inverted and placed onto dish 71 as shown in FIGURE 14 so that conical nips 90 of device 80 are seated in the conical recesses 78 of dish 71. Reagent-containing liquids are then dispensed into deposition members 87 and 88 of the assembled composite of device 80 and dish 71 by inserting a micropipette containing the liquid for a selected deposition member into the aperture 86 associated therewith and contacting the upper surface of said deposition member with the micropipette.

FIGURE 15 shows a sectional view through a portion of the assembled deposition device 80 and dish 71 before a reagent-containing liquid is added to the deposition member 88 illustrated. Peripheral portion 84 of device 80 rests on marginal portion 72 of dish 71 at a common plane 94 and deposition member 88 is spaced from gel layer 76 by the recession of depressed area 82 of device 80 and the recession of depressed central area 74 of dish 71 away from said common plane.

FIGURE 16 shows deposition member 88 in an expanded state after absorption of a reagent-containing liquid from a micropipette 92. The deposition member 88 is shown in intimate contact with gel layer 76 thereby permitting the reagent to diffuse into said gel layer. The formation of precipitin lines can take place in substantially the same manner as described with respect to the device of FIGURE 5 since the deposition members of the device 80 are arranged and shaped similarly to those in pattern 40 of the device 30.

The deposition device of the present invention can be constructed of glass, metal, or a thermoplastic polymeric material, the latter being preferred due to its moldability by either vacuum forming processes or injection molding processes. Autoclavable polycarbonate as well as other high softening point polymers are advantageous. The preferred bibulous material members are constructed of a compressed regenerated cellulose sponge. While various fibers or woven fabrics may be included in the sponge structure it has been found preferable to use only the regenerated cellulose sponge material without materials which are not fluid expandable being included therein. The deposition device will function, in a less preferred manner, by employing bibulous deposition members constructed of paper, fabric or other materials which are not fluid expandable, provided precise contact of said members with the gel layer is afforded by the device when assembled to the gel-containing dish.

The preferred cellulose sponge is precompressed such that when sufficient liquid is absorbed thereby expansion in the direction of compression, i.e., thickness, occurs which increases the thickness at least 50 percent of the compressed thickness and may increase said thickness to more than 600 percent of the compressed thickness. A suitable compressible sponge material is described in U.S. Patent No. 3,068,545 for use with various fibers. The compressed cellulose sponge material with or without the fibers can be used for the bibulous deposition members of this invention, however the cellulose sponge alone is preferred.

In the use of deposition devices constructed in accordance with the invention, excellent results have been obtained when the thickness dimension of the compressed sponge material was about one millimeter when dry and when saturated with a liquid expandable to slightly more than three millimeters, and the spacing of the dry deposition members from the gel layer was two millimeters.

The bibulous deposition members can be secured to the carrier member of the deposition device by well defined gluing patterns with a water insoluble glue or may be fusion bonded during the process of forming the device. They can also be secured by mechanical attachment means or may be wedged into indentations in the carrier

member. An accurate template should be employed to insure precise positioning of the bibulous deposition members with respect to each other in the arrangement selected.

Reagent deposition devices 30 and 80 have been described for use with mating dishes with which they form unitary devices for conducting tests. The deposition devices of this invention may, however, be made of a size and shape permitting use with standard dishes if desired.

The reagent deposition device will function adequately with only a single bibulous deposition member if a reagent is included in the gel layer. In such a case when a reagent-containing liquid is supplied to the deposition member and it expands into contact with the gel layer, a precipitation reaction will form a ring about the area of the gel layer contacted, providing test information concerning the interaction of the two reagents. It is preferable, however, to employ a plurality of deposition members in the deposition devices of this invention so that they may be used for multiple reagent studies as well as for single reagent testing against a reagent contained in a gel layer.

The reagents deposited by the device of this invention may, if desired, be pre-impregnated into the bibulous deposition members and reconstituted by the addition of water. In this dry impregnated form most reagents are stable and may be easily stored and shipped.

The registering recesses 28 and 78 in dishes 20 and 70 respectively advantageously permit the use with said dishes of templates having cooperative registering means. Such templates may be made of transparent material on which precipitin line shapes and sizes are marked which may be used as standards for interpretation of test results. Such templates can be made by the user of the deposition devices by employing standardized reagents in fresh deposition devices to form patterns in fresh gel layers and tracing the precipitin lines thus formed in the gel layers on the unmarked templates.

If desired, water impermeable films may be secured over the upper and lower plan surfaces of dishes 20 and 71 and deposition devices 30 and 80. These aid in storage and shipping and are preferably adhered so that they can be stripped off of the devices for use. Polyethylene (glycol) terephthalate film is a preferred material for this employment.

While the device of the present invention has been described primarily for use in depositing immunological reagents onto gel layer surfaces, it will be appreciated that they are capable of depositing many different organic and inorganic materials in liquid mixtures onto a variety of surfaces.

What is claimed is:

1. A deposition device comprising, a carrier member formed with at least one aperture therethrough, a bibulous material deposition member secured to one side of said carrier member in covering relation with respect to at least a portion of the area of said aperture and having a surface portion spaced from said carrier, and means defining a receiving surface, said receiving surface being disposed in juxtaposed spaced facing relation to said deposition member surface portion.

2. The deposition device of claim 1 wherein said bibulous material deposition member is fluid expandable.

3. The deposition device of claim 1 wherein said deposition member is at least 50 percent expandable in a direction away from said carrier member when wetted with a liquid.

4. The deposition device of claim 1 wherein said aperture has an enlarged end opening on the side of said carrier member opposite the side to which said deposition member is secured.

5. The deposition device of claim 1 wherein said car-

rier member is formed with at least a second aperture therethrough spaced from said first aperture, and a bibulous material deposition member is secured to said one side of said carrier member in covering relation with respect to at least the major portion of the area of said second aperture.

6. The deposition device of claim 5 wherein said first deposition member has a main body portion and a peripheral apex portion integral therewith, said apex portion spaced closer to said second deposition member than said main body portion.

7. The deposition device of claim 1 wherein said carrier member is formed with a plurality of apertures therethrough arranged about said first aperture, and a plurality of bibulous material deposition members are secured to said one side of said carrier member in covering relation with respect to at least the major portion of the area of each of said plurality of apertures respectively.

8. The deposition device of claim 7 wherein said plurality of apertures are spaced generally equidistant from said first aperture and from one another.

9. A deposition device for depositing a reagent-containing liquid onto a receiving surface comprising a carrier member and at least one deposition member of fluid expandable bibulous material secured to a surface of said carrier, said deposition member being expandable in a direction away from said carrier member surface when wetted with a liquid.

10. The deposition device of claim 9, wherein said carrier member is formed with an aperture therethrough which is at least partially covered by said deposition member.

11. The deposition device of claim 9, wherein said deposition member is at least 50 percent expandable in a direction away from said carrier member when wetted with a liquid.

12. The deposition device of claim 9, wherein at least a second deposition member of fluid expandable bibulous material is secured to said surface of said carrier at a position spaced from said first deposition member.

13. The deposition device of claim 12, wherein said first deposition member has a main body portion and at least one peripheral apex portion integral therewith, said said apex portion spaced closer to said second deposition member than said main body portion.

14. The deposition device of claim 9 in combination with means defining a receiving surface and support means therefor, said bibulous deposition member being positioned in spaced relation with respect to said receiving surface and being expandable into contact with said receiving surface.

15. The combination of claim 14 wherein there is means affording registration of said carrier member and said support means.

References Cited

UNITED STATES PATENTS

2,672,431	3/1954	Goetz	195—139
2,672,432	3/1954	Goetz	195—139
2,785,057	3/1957	Schwab et al.	23—253
2,837,055	6/1958	Whitehead.	
3,299,464	1/1967	O'Brien et al.	15—104,94
3,378,347	4/1968	Saravis	23—253

OTHER REFERENCES

Crowle, A. J., "Immunodiffusion," pp. 202-207 (1961).

JOSEPH SCOVORONEK, Primary Examiner

U.S. Cl. X.R.

195—103.5, 127; 204—180, 299