Sept. 12, 1972 G. H. BROWN ET AL 3,691,017

MEANS AND METHOD FOR CHEMICAL ANALYSIS

Filed May 6, 1970 2 Sheets-Sheet 1

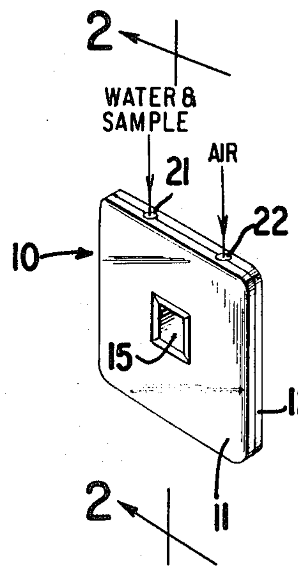

FIG. 1

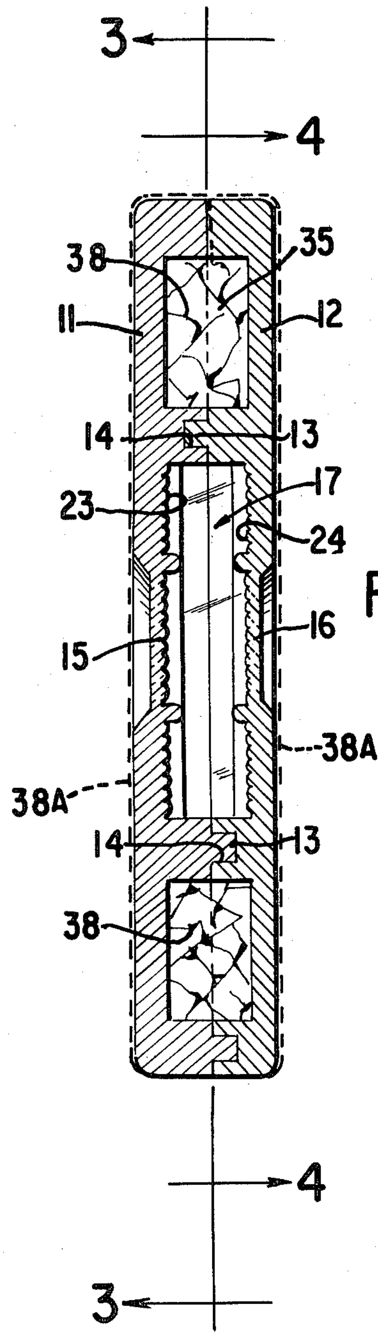

FIG. 2

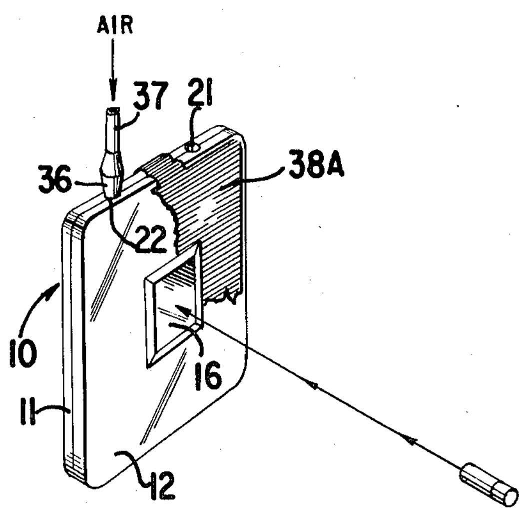

FIG. 7

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FIG.3

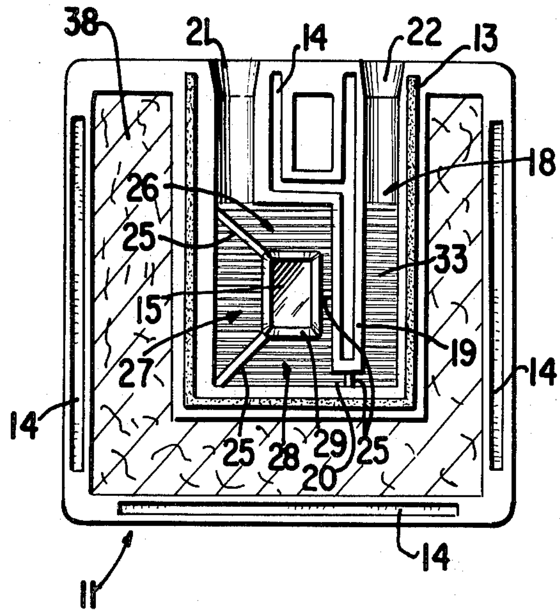
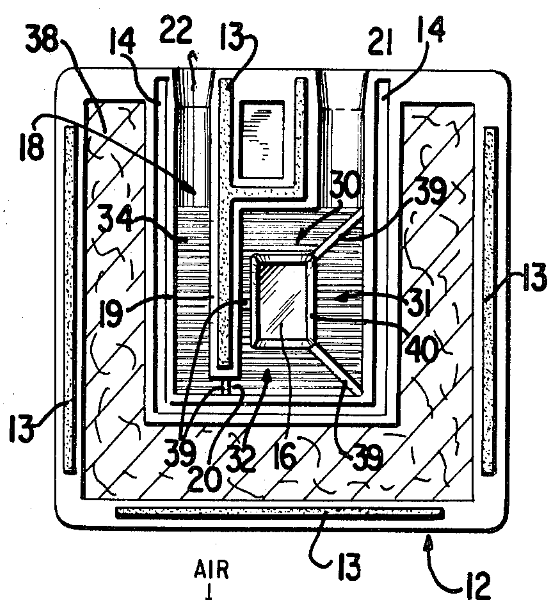


FIG.4



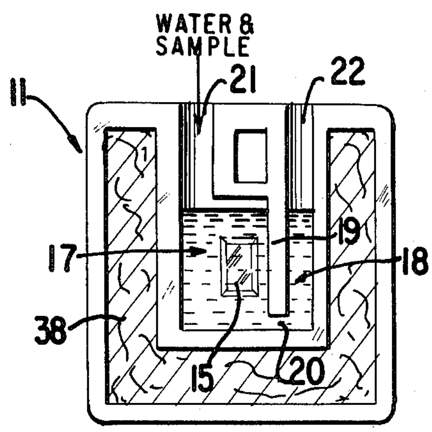


FIG.5

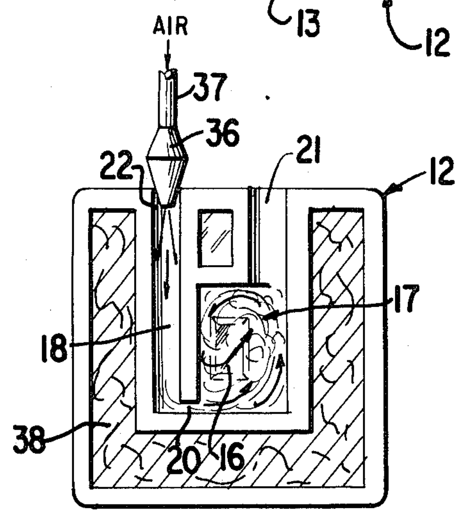


FIG.6

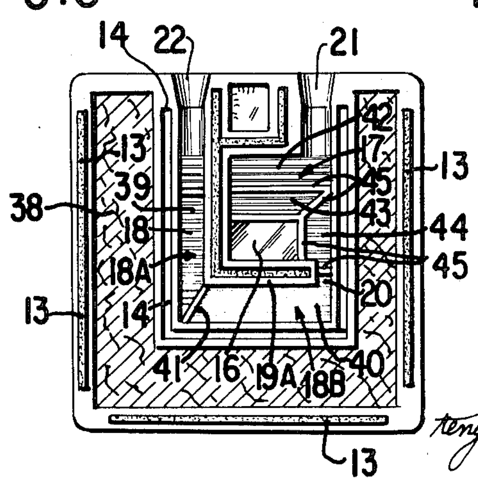


FIG.8

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MEANS AND METHOD FOR CHEMICAL ANALYSIS

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U.S. Cl. 195—103.5 R 25 Claims

ABSTRACT OF THE DISCLOSURE

A rigid or semi-rigid disposable reaction container is disclosed for use in measuring the extent or rate of optical density change produced by a reaction, which container is provided with chamber means that preferably comprises a windowed chamber and an auxiliary chamber having openings communicating with the interior thereof and interconnected by a passage of small cross-section through which solution from the auxiliary chamber containing a dissolved key reactant may be injected into the reaction chamber to initiate the chemical reaction. There is further disclosure of a disposable reaction container that is in the form of a chambered slide wherein the opening or openings providing access to the chamber means preferably occurs in the margin of the slide. There also is disclosed a disposable reaction container in the form of separable sections which are adapted to have components of the reaction mixture deposited thereon in independent separated relation on predetermined surface areas, the components being deposited in solid form preferably by lyophilization before bringing the sections of the container together in face-to-face abutting secured relation and means being provided conducive to keeping the deposited components separate from each other in the common chamber. The disclosure also describes certain procedures including the loading of the sections of the reaction container with the separated quantities of solid components of the reaction mixture followed by uniting of the sections in face-to-face relation and thereafter, when making an analysis, introducing a suitable solvent and any other component or components of the reaction mixture, the reaction being initiated by the injection of a key component. For purposes of incubation, the disposable reaction container preferably comprises an incubation chamber which is used in controlling temperature as by placing therein a metal which is responsive to a thermostatically controlled field for the generation of heat.

THE FIELD OF THE INVENTION

This invention relates to method and means for chemical analysis and relates more especially to such method and means which lends itself to automated or semi-automated analysis procedures. The invention is especially suited for use in connection with the analysis of body fluids such as blood, urine, and the like.

BACKGROUND OF THE INVENTION

Especially in the field of biochemistry, there is a very extensive need for chemical testing from the point of view of both quantitative and qualitative analysis pertaining to substances known to have relevance to possible malfunctioning of the biochemical processes of living mammals. For example, it has been estimated that the number of blood tests run in the United States during 1969 was substantially in excess of one billion and may be doubled in the near future. When one takes into account the vast number of tests that are comprehended by multiphasic screening, diagnostic testing and patient monitoring, it becomes apparent that the existing testing facilities are heavily taxed both as regards equipment and personnel. There have been a number of proposals for automatic or semi-automatic testing equipment and procedures. However, proposals heretofore made have had inherent shortcomings such as complexity and cost, less than desired accuracy, limited flexibility and the necessity for the employment of trained technical personnel.

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OBJECTS OF THE INVENTION

A principal object of this invention is to provide simple and inexpensive means for performing analyses of the character aforesaid with a high degree of accuracy and without the necessity for employing highly trained technical personnel.

It is a further object of this invention to provide a disposable reaction container which is simple, made of inexpensive material and devoid of moving parts that lends itself to automated handling in the measurement of optical density relative to the transmission of light through a predetermined thickness of the reaction mass in the disposable container.

A further object of this invention is to provide a disposable reaction container that also serves as a vehicle for the separated storage of reactants in solid form in common chamber means.

It is a further object of this invention to provide a disposable reaction container which is constructed so as to enable a chemical reaction to be initiated by the introduction of a key component at a desired time.

It is a further object of this invention to provide a disposable reaction container which comprises means whereby after all components of the reaction mass have been placed in the reaction container the reaction mass may be brought to desired temperature and maintained at said temperature during a period of incubation.

THE GENERAL NATURE OF THE INVENTION

This invention involves the employment of a disposable reaction container which is composed of rigid or semi-rigid material and which comprises transparent windows in predetermined spaced relationship to each other for the successive transmission of light therethrough and through a layer of predetermined thickness of the reaction mass within the container. When the reference is to transparent windows the reference is general to the transmission of light whether or not in the visible range. For example, for analyses such as those principally contemplated herein it is common practice to employ a spectrophotometer as a source of monochromatic light in the wave length range from 330 mμ to 600 mμ. One wave length which is commonly used is in ultraviolet light at a wave length of essentially 340 mμ.

The disposable reaction container comprises chamber means which not only is provided with the transparent windows but also is provided with areas that are adapted for the deposition thereon of reaction components in solid form and that in the finished commercial product does have soluble reactants in solid form deposited thereon in out-of-contact relation. These areas preferably have ribs between them which protrude inwardly into the reaction chamber but do not extend across the width thereof, thereby facilitating the deposit of the solid reaction components in the separated state so as to minimize the possibility of their deterioration caused by any premature commingling of the components. Moreover, each of the areas preferably is provided with a multiplicity of protrusions which assist in maintaining proper disposition of the solids within the chamber means of the reaction container and minimize flow on said surface when the components are initially applied in the form of a solution. In this way it is possible to provide as a commercial product a disposable reaction container having stored therein the various components of the reaction mass other than the solvent and other than the sample which is to be introduced in making the analysis.

It is a further feature of this invention that the reaction container is initially made in separate sections so as to facilitate the deposition of the solid components at the desired areas within the chamber means of the reaction container. In typical practice of the invention solutions containing predetermined quantities of dissolved solid reaction components are deposited on selected areas of the sections and the reaction components are reduced to solid form by lyophilization. After this has been accomplished the sections are united in assembled relation. In normal construction each section comprises one of the transparent windows and the sections are brought into face-to-face relationship. Any suitable uniting means may be employed for holding them in position. Preferably the uniting means that is employed comprises relatively movable surfaces in frictional contacting relationship so that the sections may be secured together merely by the application of pressure. The employment of tongue and groove interfitting parts is well suited for accomplishing a press fit and frictional holding of the sections together. An adhesive may be used additionally or alternatively but ordinarily an adhesive is unnecessary.

A further feature of the invention resides in the employment of a chambered slide comprising interior chamber means provided with an opening or openings communicating with the interior thereof. Preferably the opening or openings are located at the margin of the slide. This construction coupled with the employment of rigid or semi-rigid material for the reaction container enables the reaction container to be handled as a relatively thin slide or plate which can be held with the opening or openings upwardly. This configuration and construction of the disposable container enables the container to be readily handled by automated mechanisms when carrying out an analysis or test in the manner that will be described more in detail hereinbelow.

It is a further feature of this invention that the chamber means that is employed comprises a reaction chamber in combination with an auxiliary chamber. The reaction chamber has an opening therein communicating with the interior thereof through which the solvent and the sample may be introduced. The auxiliary chamber also has an opening communicating with the interior thereof that is upwardly disposed when the reaction container is in position with the opening for the reaction chamber upwardly disposed. The reaction chamber and the auxiliary chamber are in communication with each other through a passage of reduced cross-section which ordinarily is disposed in portions of the reaction chamber and the auxiliary chamber that are remote from the aforesaid openings. When the reaction container is in the form of a chambered slide the openings for the reaction chamber and for the auxiliary chamber preferably are disposed in adjacent relation at one of the margins of the slide. When employing chamber means comprising both a reaction chamber and an auxiliary chamber reaction components may be deposited on certain areas of the interior surface of the reaction chamber and another and different reaction component may be deposited on the interior surface of the auxiliary chamber. The substance deposited on a surface presented by the auxiliary chamber preferably is a substance which is required in order to initiate or trigger the chemical reaction. For purposes of brevity, a component responding to this requirement is referred to sometimes hereinafter and in the claims as a "key component."

When the disposable reaction container is provided with chamber means comprising a reaction chamber and an auxiliary chamber as previously described, the reaction components other than the solvent and the sample or specimen to be tested are provided in solid form in the reaction chamber and in the auxiliary chamber as hereinabove described. When it is desired to make an analysis a solvent such as water is introduced into the reaction chamber in a predetermined amount in relation to the capacity of the reaction chamber and of the auxiliary chamber such that the windows in the reaction chamber are covered. The sample thereafter is added and since the solvent previously has flowed into the auxiliary chamber very little or none of the component in the auxiliary chamber upon becoming dissolved migrates into the reaction chamber. When the contents are in this condition, the contents of the reaction container may be brought to desired temperature for the reaction and also may be permitted to incubate for such period of time as may be desired. When, however, it is desired to initiate the reaction, the solution of the key component in the auxiliary chamber is forced therefrom through the restricted passage into the reaction chamber and upon the injection of the solution of the key component into the reaction chamber from the auxiliary chamber it becomes commingled with the solution of the other components in the reaction chamber, including the sample, and the reaction is initiated. The ribs in the reaction chamber and the protrusions hereinabove mentioned assist in setting up turbulence during the injection. In order to insure thorough and complete commingling of the reactants, the solution in the mixing chamber may be caused to flow back into the auxiliary chamber whereupon the injection may be repeated several times.

The forceful ejection of the solution in the auxiliary chamber through the passage of reduced cross-section, preferably is accomplished by the employment of a puff of compressed air. To this end, the opening into the auxiliary chamber may be round for the reception of a nozzle having a conical exterior surface at the end of a line which supplies the puff of compressed air. The return of solution into the auxiliary chamber may be accomplished by the pressure exerted by gravity. However, such pressure may be assisted either by subjecting the interior of the auxiliary chamber to a short interval of subatmospheric pressure exerted through the opening and nozzle of an air line into the opening into the reaction chamber, which opening in such case ordinarily would be round for the reception of a nozzle having an exterior cone-shaped surface. The insertion of the nozzle in either opening may be accomplished manually. However, this invention contemplates the handling of the chambered slides by equipment such that at the appropriate time the nozzle or nozzles are brought into operative relation with the opening or openings in the slide followed by their disengagement when the desired mixing has been accomplished.

It is a further feature of preferred practice of this invention to include in the disposable reaction container, incubation means comprising a chamber which serves the purpose of bringing the reaction mixture to a desired temperature during the period of incubation before initiating the reaction. In preferred practice of the invention the incubation chamber may be one which is disposed in contiguous relation with the other chamber means and it may contain a metal distributed therethrough which when heated assists in bringing the reaction container and its contents to desired temperature and in maintaining desired temperature. The metal within the container may be heated by conduction, from a continuation of the metal extending to the exterior that is heated as by contact with a heated holder or air or in response to energy received from an energizing field such as a high frequency electric field.

In carrying out the method of the invention, the disposable reaction chamber is initially made up so as to contain solid components of the reaction mixture preferably deposited by lyophilization in situ on the surfaces of the sections of the container before they are united with each other. Because of the construction and configuration of the surface areas of the chamber means a number of different reaction components may be deposited in the container in solid form and in predetermined quantities appropriate for the contemplated analysis. As will be illustrated more concretely hereinafter, the chamber means of a chambered slide may readily present as many as eight or more individual surfaces on which components may be deposited in solid form. The particular components that are employed in any case depends, of course, on the nature of the analysis that is to be performed. It is one of the advantages of this invention that it has great flexibility in its capacity to accommodate to an automatic or semi-automatic procedure a wide variety of analyses and tests notwithstanding very great differences in the nature and number of reaction components.

When it is desired to use the reaction container in the form wherein the chamber means includes an auxiliary chamber, all that is required is to add a predetermined amount of water and the sample, e.g. a small quantity of blood serum. The container may desirably be disposed in automatic equipment with the openings disposed upwardly for the reception of the solvent and the sample. After introduction of the solvent and the sample, the container which has now been charged with all of the ingredients of the reaction mass, ordinarily is brought to a predetermined temperature for carrying out the reaction. And when an incubation period is required, the desired temperature is maintained during the incubation period. After initiation of the chemical reaction by forceful injection of solution containing a key component from said auxiliary chamber into said reaction chamber, the reaction container is brought into operative relation with a beam of monochromatic light from a suitable source such as a spectrophotometer, and readings may be made which reflect optical density according to known procedures, the reaction container itself serving in effect as a cuvette. When the rate of reaction is to be determined, then optical density readings may be taken at stated intervals from which the rate of change in optical density may be computed.

While this invention lends itself to the employment of any combination of reactants and components appropriate for use in the reaction mass in the performance of tests and analyses involving reactions that have an effect on optical density, including more especially those carried out in an aqueous medium using water as the solvent, it is one of the advantageous features of this invention that it is well suited for carrying out reactions of the enzymatic type involving enzymatic reagents. Any of the reaction components normally used in such reactions may be employed such as enzymes, co-enzymes, selected substrates and such other components as may be desired such as buffers and inorganic salts.

When carrying out the invention utilizing a reaction container wherein the chamber means comprises a reaction chamber in combination with an auxiliary chamber containing the key component, and utilizing enzymatic reagents comprising a substrate, it is normally preferred that the substrate be initially deposited within the auxiliary chamber inasmuch as it is preferable to employ the substrate as the key component which initiates the reaction upon a solution thereof being injected into the reaction chamber.

While it is preferable to employ a reaction container having a windowed reaction chamber in combination with a contiguous auxiliary chamber that is separate from the reaction chamber except for a passage of reduced cross-section, it is within the purview of some of the aspects of this invention to employ only a single chamber, namely, the reaction chamber. In such case, all of the reaction components may be deposited in solid form on areas within the reaction chamber with the exception of the key component, which in this case ordinarily is the sample. When the analysis is to be performed then the solvent is introduced into the chamber and the contents of the reaction container may then be brought to desired temperature during an incubation period, as has been described hereinabove. When it is desired to initiate or trigger the reaction, a solution of the sample is introduced into the reaction chamber and mixed with the contents thereof as by the employment of a jet of air or the introduction of a stirrer. Thereafter, the extent of any effect on optical density may be observed as described hereinabove.

DETAILED DESCRIPTION OF THE INVENTION

Further objects, features and advantages of this invention will become apparent from the following description in connection with a specific embodiment of the disposable reaction container as illustrated in the accompanying drawings, wherein:

FIG. 1 is a perspective view of a disposable reaction container in the preferred form of a chambered slide;

FIG. 2 is a cross-section on an enlarged scale of the chambered slide along the line **2—2** of FIG. 1;

FIGS. 3 and 4 are, respectively, plan views of the separated sections of the chambered slide in the direction of lines **3—3** and **4—4**, respectively, as shown in FIG. 2; FIGS. 3 and 4 being on a somewhat smaller scale as compared with FIG. 2;

FIG. 5 is a schematic elevation of the slide section corresponding with FIG. 3 illustrating the liquid level that is assumed in the reaction and auxiliary chambers;

FIG. 6 is similar to FIG. 5 and illustrates schematically in connection with the complementary section of FIG. 4 the forcing of liquid from the auxiliary chamber into the reaction chamber responsive to a puff of air with resultant mixing in the reaction chamber;

FIG. 7 shows the chambered slide in perspective in relation to a source of air under pressure and to a source of light emanating from a source; and

FIG. 8 corresponds with FIG. 4 but on a smaller scale and is illustrative of an alternative chamber arrangement.

Having reference to the illustrative embodiment shown in the drawings, the disposable reaction container is shown in its preferred form, namely, in the form of a chambered slide, which is indicated generally by the reference character **10**. The slide is composed of two abutting planar sections **11** and **12** which are shown in FIG. 2 as being held in face-to-face relation by tongues **13** which interfit with grooves **14** in sliding frictional relation such that the sections may be forced together by pressure and detachably held together by the frictional contact between the interfitting surfaces of the tongues and grooves. The arrangement of parts in section **11** is shown in FIG. 3 and the arrangement of parts in section **12** is shown in FIG. 4. As is apparent from the drawings, the arrangements are complementary so that the different portions of each section oppose each other when the sections are brought together as shown in FIG. 2. In the central portion of section **11** there is a transparent window **15** which is in opposed spaced relation with respect to the window **16** in section **12**.

In the central portion of the slide planar extended chamber means is provided. In the embodiment shown the larger portion of the chamber means in the region of the windows is the reaction chamber **17**. The chamber means also comprises the auxiliary chamber **18** which is separated from the reaction chamber **17** by a wall **19** except for a passageway **20** of reduced cross-section. The reaction chamber is provided with an opening **21** which communicates with the interior thereof and the auxiliary chamber **18** is provided with an opening **22** that communicates with its interior. It is to be noted that in the preferred embodiment shown in the drawings, the openings **21** and **22** are located in proximity to each other in one of the margins of the chambered slide and that when the slide is in position with one of the openings disposed upwardly, the other opening also is disposed upwardly. The passage **20** is shown as disposed according to preferred practice in the portion of the chamber means opposite to the openings **21** and **22**.

The inner surface of the section **11** that is in the vicinity of the window **15** is provided with a multiplicity of small protrusions therefrom which, as shown, are in the form of a succession of small ridges **23**. Similarly, the portion of section **12** of the slide adjacent the window **16** has small ridges **24** protruding therefrom. In section **11** the ribs **25** separate the inner surface of the chamber into three areas which are indicated by the reference characters **26**, **27** and **28**. Similar ribs **29** border the window **15'**. In section **12** the ribs **39** and **40** subdivide the area surrounding the window **16** into areas **30**, **31** and **32**. The ribs **25**, **29**, **39** and **40** protrude into the reaction chamber but do not extend thereacross.

As regards the auxiliary chamber **18**, the inner surface likewise presents protrusions **33** in the form of small ridges.

When the sections **11** and **12** are secured in face-to-face relationship, the tongue and groove jointure about the chamber means provides means to retain a liquid therein when the openings **21** and **22** are disposed upwardly.

In the preferred embodiment shown, there is an additional chamber **35** which borders the central chamber means which holds the reaction mass. The chamber **35** is referred to herein and in the claims as an incubation chamber. It provides means that is part of the reaction container itself whereby the reaction mass after its completion, except for the commingling of the key component with the other components, may be brought to desired temperature and maintained at desired temperature during an incubation period. In the embodiment shown the chamber **35** has metal disposed therein which conveniently may be aluminum foil **38**. Aluminum is a metal which is a good conductor of heat and which when subjected to an energized field such as high frequency electricity, absorbs energy in the form of heat. These properties may be utilized in bringing the slide and its contents to desired temperature and in maintaining desired temperature. In the embodiment shown a continuation **38A** of the aluminum foil within the chamber **30** is extended so as to overlie a substantial portion of the external surface of the slide other than the windows. When utilizing the slide, it is contemplated that a plurality of the slides will be disposed in a suitable holder which may be made of aluminum and which by its disposition in an environment maintained at the desired temperature becomes heated to substantially the same temperature. In such case, the aluminum foil overlying the external surface of the slide receives heat from the environment and likewise as the result of contact with surfaces of the holder. The heat so received becomes transmitted by conduction to the foil within the chamber **35** so that the container and its contents may be brought to desired temperature more quickly. Alternatively, the aluminum foil may be heated by subjecting it to a high frequency electric field. In such case, automatic control may be provided by thermostatic control which energizes and de-energises the energizing field according to whether the temperature goes below or above the predetermined temperature to be maintained. The external continuation **38A** of the foil may be employed to facilitate detection of the temperature of the aluminum foil. Temperature control also may be had in other ways. Thus the chamber **35** may be omitted and the desired temperature during incubation may be obtained by partial immersion of the slide in a body of liquid maintained at a desired temperature. Alternatively, if desired, the metal foil may be omitted from the chamber **35** and by the employment of suitable inlet means and outlet means (not shown) for the chamber **35** a fluid at a desired predetermined temperature may be flowed through the chamber **35** during the incubation period.

Typical usage of the chambered slide is illustrated in connection with the following examples:

Example 1

According to this example, the chambered slide of this invention as illustrated in the drawings is employed in an analysis for the determination of the amount of lactic dehydrogenase in a sample of blood serum. When a person become afflicted with the heart condition known as myocardial infarction the body releases certain enzymes into the blood stream. One of the enzymes so released is lactic dehydrogenase and the presence of a more than normal amount of lactic dehydrogenase in a sample of blood serum is symptomatic of a myocardial infarction condition. The reagents employed in an analysis for the detection of lactic dehydrogenase are lactic acid and diphosphopyridine nucleotide (DPN) and a phosphate buffer.

Having specific reference to the chambered slide shown in the drawings, the components of the reaction mixture other than the water and the sample are first deposited on the desired surface areas of the slide while the sections **11** and **12** are in the separated condition as illustrated in FIGS. 3 and 4. In the reaction under present consideration, the lactic acid is the substrate and is the key component which initiates or triggers the reaction. The lactic acid is deposited from solution on a surface area presented in the auxiliary chamber **18**, namely, the surface **33** of section **11** or the surface **34** of section **12**, or both of these surfaces. A solution of DPN may be deposited on area **26** of section **11** or area **30** of section **12**, or both. Buffered solution may be applied to sections **27** or **28** or both of section **11** or sections **31** and **33** or both of section **12** or on all four of these areas. After the solutions have been deposited on the respective areas above mentioned, the solutions are reduced to solid form, preferably by lyophilization. Each of the reagents is present in the predetermined amount for providing the desired concentration thereof in the reaction mass when it is completed by the addition of water and the sample.

In typical practice of the invention, the chambered slide may be 2 inches square and 5/16 inch in thickness, the distance between the inner surface of the windows being 5 mm. The dimensions of the windows are 1/2 inch by 1/4 inch. The diameter of the round opening communicating with the interior of the reaction chamber is 1/8 inch and the diameter of the round opening communicating with the interior of the auxiliary chamber is 1/8 inch and the dimensions of the passageway **20** are 1/16 inch by 2.5 mm. The capacity of the chamber means is such that 1.4 ml. of water or other solvent fills the chamber means to the level indicated in FIG. 5. The slide may be made of any rigid or semi-rigid material such as a styrene or a clear acrylic resin subject to the windows having adequate transparency for the monochromatic light used for the contemplated test. By rigid or semi-rigid, it is meant that the container structure have sufficient rigidity so that the distance between the windows is substantially accurately maintained at a predetermined spacing.

When the reaction components have been reduced to solid form, they adhere to the different areas and remain separated from each other and when a metal such as aluminum foil is employed in the incubation chamber, it is put in place in one of the sections. The sections are then brought to the position shown in FIG. 2, thereby completing the slide as a commercial product. The completed slide may be used immediately or packed in boxes for shipment and storage prior to use by a customer.

When the analysis is to be performed the operator adds 1.4 ml. of water through the opening **21**. During this step the added water flows into the auxiliary chamber. Thereafter 0.1 ml. of serum sample is introduced into the reaction chamber. These additions may be accomplished in any suitable way, as by the employment of a micropipette or a microsyringe. However, as regards the water addition, the correct quantity of water can be measured into the slide when the amount required to fill to an indicated level is known. After the addition of the water and the serum sample, the slide and its contents are brought to a constant temperature which, for the illustrative test in question, is from 30° to 37° C., depending on the setting that may be desired in this range. For a given test the temperature should be brought within ±0.2° C. of the reaction temperature that is desired. This ordinarily requires about one minute, and to accomplish desired incubation the reaction mass may be maintained at this temperature for an additional 4 minutes. When it is desired to initiate the reaction the opening **22** into the auxiliary chamber is brought into operative relation with the nozzle **36** of an air line **37** and the reaction is initiated by introducing into the auxiliary chamber a small puff of air such as .5 cc. which forces the contents of the auxiliary chamber through the restricted passage **20** into the reaction chamber **17** with a swirling action, as illustrated in FIG. 6. When this operation is performed manually a short length of resilient rubber hose may be used that has a conical nozzle at the end thereof that fits into the opening **22** of the auxiliary chamber and the puff of air may be produced by squeezing the rubber hose. The mouth of the opening **22** may be somewhat flared so as to conform to the conical surface of the nozzle **36**. The presence of the ribs protruding from the surfaces of the reaction chamber assist in the mixing action. If desired, air pressure in the auxiliary chamber. Alternatively, a mechanical plunger could be restored, whereupon a second puff of air may be employed to further the mixing action in the mixing chamber. If desired, this may be repeated and more generally the flow back and forth through the restricted passage **20** may be effected by subatmospheric or superatmospheric pressure applied to either the auxiliary chamber or to the reaction chamber. Alternatively, a mechanical punger could be employed instead of air.

When the mixing of the reaction mixture has been completed, any lactic dehydrogenase in the blood sample acts as a catalyst which catalyzes the reaction to form as reaction products pyruvic acid and DPN in the reduced form (DPNH). Since the DPNH, which is produced as a reaction product, has a substantially higher optical density for light at 340 mμ than does DPN, the rate of any increase in optical density is a function of the amount of lactic dehydrogenase in the sample of blood serum. After the initiation of the reaction as the result of commingling the substrate with the other reaction components, the reaction rate may be determined by moving the slide into position for the passage of light at 340 mμ through the transparent windows of the slide and through the thickness of the reaction mass between the two windows which, for the purposes of this example, may be 5 mm. Any change in optical density per unit of time may be measured and in known fashion the data thus obtained may be translated into values indicative of the quantity of lactic dehydrogenase contained in the sample of blood serum. In an analysis such as that exemplified, ten readings at intervals of one minute are adequate.

Example 2

The practice of this invention may be further illustrated by its employment in the determination of creatine phosphokinase (CPK). The presence of CPK in blood serum is generally indicative of muscular disease. The reagents employed are creatine, adenosine triphosphate, glutathione, phosphoenol pyruvate, pyruvate kinase, lactate dehydrogenase, reduced diphosphopyridine nucleotide (DPNH) and a phosphate buffer.

In preparing the slide the creatine, which is a substrate and functions as the key component, is caused to be deposited in solid form on the surface area within the auxiliary chamber. The other components may be deposited in any convenient way on surface areas that are presented in the reaction chamber. If desired, certain of the compatible

reaction components may be combined such as the glutathione with the phosphate buffer. The two enzymes may likewise be combined. When the analysis is to be carried out, water is added followed by a sample of blood serum, and the slide and its contents are brought to desired reaction temperature and are subjected to incubation, as above described in connection with Example 1, followed by initiation of the reaction by the injection of the solution of creatine into the reaction chamber and the making of optical density readings at stated intervals, as also described in connection with Example 1. When the reaction mixture is completed the reaction of creatine and adenosine triphosphate is catalyzed by any creatine phosphokinase in the blood serum with the production of creatine phosphate and adenosine diphosphate. Pyruvate kinase in turn catalyzes the reaction of adenosine diphosphate with phosphoenol pyruvate with reformation of adenosine triphosphate and pyruvate which is thus made available for the indicator reaction wherein the lactate dehydrogenase catalyzes the reaction between pyruvate and the reduced diphosphopyridine nucleotide (DPNH) with resultant formation of lactate and diphosphopyridine nucleotide (DPN) which has less optical density for monochromatic light at 340 mμ than DPNH. In this analysis successively timed determinations of decreasing optical density are indicative of the rate of the reaction and in turn the concentration of creatine phosphokinase in the sample of blood serum.

It is to be understood that the foregoing is solely for the purpose of illustrating a typical embodiment and utilization of the invention. Thus the reaction container may occur in other forms or in other configurations within the purview of the invention as herein disclosed and claimed. One such modification is illustrated in FIG. 8 which is generally similar to FIG. 4 wherein the parts which are essentially the same are indicated by like reference characters. The principal modification which distinguishes FIG. 8 from FIG. 4 is that the wall **19** is provided with the extension **19A** which as shown is disposed in parallelism with the bottom of the chamber means and is spaced therefrom by the buffer zone **18B** which is an extension of the primary zone **18A** of the auxiliary chamber **18**. The passage **20** of reduced cross-section is disposed as indicated in FIG. 8, i.e., at a location that is separated from primary zone **18A** by the longitudinal extent of the buffer zone **18B** which ordinarily is of substantially greater longitudinal extent than width. The auxiliary chamber **18**, including the zone **18B**, is shown as presenting the ridged surface areas **39** in zone **18A** and **40** in zone **18B** separated by the rib **41**. The reaction chamber **17** is shown as presenting the ridged surface areas **42, 43** and **44** which are separated from each other and from the window **16** by the ribs **45**.

FIG. 8 shows one section only of the complete slide. The other section may be the complement in all respects of that shown in FIG. 8. However, this is not necessarily the case. Thus, the arrangement of surface areas may be different as compared with FIG. 8, or alternatively, the surfaces presented by the interior of the auxiliary and reaction chambers may be plain when it is not regarded as necessary to deposit any of the reaction components therein.

The chamber arrangement shown in FIG. 8 may be desired to provide additional assurance that during the incubation period none of the key component migrates from the auxiliary chamber into the reaction chamber as, for example, if a prolonged incubation period is desired or if the components of the reaction mass are such as to be conducive to rapid diffusion of the key component. When utilizing the embodiment shown in FIG. 1 the key component, such as the substrate of an enzymatic reaction, ordinarily is deposited on the ridged surface of the primary zone **18A** while one or more other and different reactants is or are disposed on the ridged surface of any or all of the areas **42**, **43** and **44** presented by the interior surface of the reaction chamber. The ridged surface of the buffer zone **18B** of the auxiliary chamber may have nothing deposited thereon or may have deposited thereon some soluble component of the reaction mass, such as a buffer, that is inert with respect to the component deposited in zone **18A**. In either case, when the reaction container is put into use by the introduction of water into the reaction chamber the water flows into the auxiliary chamber via the opening **20** and the zone **18B** before entering zone **18A**. Notwithstanding solution of the key component in zone **18A**, the possibility during incubation of migration by diffusion of any of the key component from zone **18A** into the reaction chamber is doubly insured by the necessity for travel through the buffer zone **18B** as well as through the passage **20** of reduced cross-section. If the zone **18B** is not utilized for the deposition therein of a soluble solid component of the reaction mass, the buffer zone **18B** may approach the cross-sectional dimensions of the passage **20** of restricted cross-section and thereby provide an elongated passage which may have substantially the same restricted cross-sectional dimensions throughout its length.

While the opposed sections of the reaction container have been illustrated as utilizing tongue and groove members adapted to frictionally hold the sections together and provide fluid-tight peripheral walls for the auxiliary and reaction chambers and provide a fluid-tight wall between the auxiliary and reaction chambers, other means may be provided. Thus, if desired, prevention of accidental separation may be assured by the employment of conventional cooperating latch members that resiliently snap into locked position when the opposing sections are pressed together.

It is to be understood that the foregoing examples are merely illustrative of the flexibility and adaptability of the invention to different tests and analyses and to automated diagnostic and analytic procedures.

We claim:

1. A disposable reaction container which is composed essentially of rigid or semi-rigid material and the walls of which present interior surfaces that define

a reaction chamber, and

an auxiliary chamber,

said container having

a first opening therein that is in communication with the interior of said reaction chamber,

a second opening that is in communication with the interior of said auxiliary chamber and that is upwardly disposed when said container is in position with said first opening upwardly disposed,

said container having a passage of restricted cross-section communicating between said reaction chamber and said auxiliary chamber adjacent the portions thereof remote from said first and second openings, said passage being adapted and arranged for the injection of liquid from said auxiliary chamber into said reaction chamber so as to become commingled with liquid in said reaction chamber upon forcing liquid from said auxiliary chamber through said passage,

said reaction chamber having transparent windows therein adapted and arranged for the transmission of light therethrough and through a body of predetermined thickness of liquid in said reaction chamber between said windows, and

said auxiliary chamber and said second opening in communication therewith being adapted for being placed in operative relation with a source of gas under pressure and for imposing gas pressure supplied from said source on liquid in said auxiliary chamber for forcing it into said reaction chamber through said passage.

2. A disposable reaction container according to claim **1** wherein a first soluble reaction component in solid form is within said reaction chamber and a second soluble reaction component in solid form that is different from said first component is within said auxiliary chamber.

3. A disposable reaction container according to claim **2** wherein said second component is a key reaction component.

4. A disposable reaction container according to claim **2** wherein said second reaction component is a substrate for an enzymatically induced reaction with said first reaction component.

5. A disposable reaction container which is composed essentially of rigid or semi-rigid material and the walls of which present interior surfaces that define

a reaction chamber, and

an auxiliary chamber,

said container having

a first opening therein that is in communication with the interior of said reaction chamber,

a second opening that is in communication with the interior of said auxiliary chamber and that is upwardly disposed when said container is in position with said first opening upwardly disposed,

said container having a passage of restricted cross-section communicating between said reaction chamber and said auxiliary chamber adjacent the portions thereof remote from said first and second openings, said passage being adapted and arranged for the injection of liquid from said auxiliary chamber into said reaction chamber so as to become commingled with liquid in said reaction chamber upon forcing liquid from said auxiliary chamber through said passage,

said reaction chamber having transparent windows therein adapted and arranged for the transmission of light therethrough and through a body of predetermined thickness of liquid in said reaction chamber between said windows, and

said container comprising as an integral part thereof incubating means comprising a chamber that is in contiguous thermally-conductive but non-interconnected relation with respect to said reaction chamber and with respect to said auxiliary chamber and that is adapted to contain a substance heated to a desired incubating temperature whereby the contents of said chambers may be heated to and maintained at said incubating temperature.

6. A disposable reaction container according to claim **5** wherein said incubating chamber contains metal adapted to be heated responsive to an energy-imparting electric field.

7. A disposable reaction container according to claim **5** wherein said incubating chamber contains a heat-conductive metal and wherein said metal has a continuation thereof of substantial extent disposed on the exterior of said reaction container.

8. A disposable reagent-containing reaction container in the form of a chambered slide which is composed essentially of rigid or semi-rigid material and which comprises interior planar extended chamber means having an opening communicating with the interior thereof,

said slide comprising planar sections in abutting face-to-face relation,

a transparent window in each of said sections, said windows being on opposite sides of said chamber means and adapted and arranged for the successive transmission of light therethrough and through a portion of the chamber means therebetween,

means securing said sections together in face-to-face fluid-tight relation for the retention in said chamber means when said opening is disposed upwardly of liquid introduced into said chamber means through said opening, and

a surface area presented by at least one of said sections having deposited thereon in solid form a predetermined quantity of a soluble reaction component.

9. A disposable reactant-containing reaction container according to claim **8** wherein said soluble reaction component is deposited in solid form on said area by lyophilization in situ.

10. A disposable reactant-containing reaction container according to claim **8** wherein the surface of said area having said reaction component deposited therein presents a multiplicity of protrusions which are restrictive of flow of liquid along said surface.

11. A disposable reactant-containing reaction container according to claim **8** wherein said chamber means presents a plurality of surface areas having different reaction components in predetermined quantity deposited thereon.

12. A disposable reaction container in the form of a chambered slide which is composed essentially of rigid or semi-rigid material and which comprises an internal planar extended reaction chamber having an opening communicating with the interior thereof,

said slide presenting planar sections in abutting face-to-face relation,

a transparent window in each of said sections, said windows being on opposite sides of said reaction chamber and adapted and arranged for the successive transmission of light through a portion of the reaction chamber therebetween,

means securing said sections together in said abutting face-to-face relation and in fluid-tight relation for the retention in said chamber when said opening is disposed upwardly of liquid introduced into said chamber through said opening, and

at least one of said sections presenting a plurality of surface areas adjoining each other and the window therein, adjoining areas being separated from each other by a rib that projects into and only partially across the width of said reaction chamber.

13. A disposable reaction container in the form of a chambered slide which is composed essentially of rigid or semi-rigid material and which comprises

a planar extended reaction chamber having an opening therein affording communication with the interior thereof,

incubating means comprising a chamber laterally disposed with respect to the periphery of said reaction chamber in contiguous thermally-conductive non-interconnected relation therewith,

said slide presenting abutting planar sections in complementary face-to-face abutting relations,

a transparent window in each of said sections, said windows being on opposite sides of said reaction chamber and adapted and arranged for the transmission of light successively therethrough and through a portion of the reaction chamber therebetween,

means securing said sections in face-to-face abutting relation for retention in said reaction chamber when said opening is disposed upwardly of liquid introduced into said chamber through said opening, and

said chamber comprised in said incubating means being adapted to contain a heated substance whereby the contents of said reaction chamber may be heated and maintained at a desired incubating temperature.

14. A disposable reaction container according to claim **13** wherein said incubating chamber contains metal adapted to be heated.

15. A disposable reaction container in the form of a chambered slide composed essentially of rigid or semi-rigid material, said slide comprising

a planar extended reaction chamber,

a first opening in the margin of said slide in open communication with the interior of said reaction chamber,

an auxiliary planar extended chamber laterally adjacent said reaction chamber and separated from said reaction chamber except for a passage of reduced cross-section communicating between said chambers adjacent the portions thereof remote from said margin of said slide having said opening therein,

a second opening in said margin of said slide that is separated from said first opening by a wall that separates said reaction chamber from said auxiliary chamber except for said passage, said second opening being in proximate spaced relation with respect to said first opening and in open communication with the interior of said auxiliary chamber, and

a transparent laterally disposed window in each wall of said reaction chamber, each window being in spaced relation relative to the other and said windows being disposed for the successive transfer of light therethrough and through the portion of the reaction chamber therebetween.

said chambered slide comprising abutting planar sections in complementary face-to-face relation, the inner surfaces of which define said reaction and auxiliary chambers and each of which has one of said windows therein, and

securing means which secures said sections together in face-to-face liquid-tight relation for the retention of liquid therein when said openings in said slide are disposed upwardly.

16. A disposable reaction container according to claim **15** wherein said securing means comprises opposing surfaces in frictional detachable engagement.

17. A disposable reaction container according to claim **15** wherein said securing means comprises cooperating latching members.

18. A disposable reaction container in the form of a chambered slide composed essentially of rigid or semi-rigid material, said slide comprising

a planar extended reaction chamber,

a first opening in the margin of said slide in open communication with the interior of said reaction chamber

an auxiliary planar extended chamber laterally adjacent said reaction chamber and separated from said reaction chamber except for a passage of reduced cross-section communicating between said chambers adjacent the portions thereof remote from said margin of said slide having said opening therein,

a second opening in said margin of said slide in proximate spaced relation with respect to said first opening and in open communication with the interior of said auxiliary chamber, and

a transparent laterally disposed window each wall of said reaction chamber, each window being in spaced relation relative to the other and said windows being disposed for the successive transfer of light therethrough and through the portion of the reaction chamber therebetween, and

at least one of said sections presenting a surface area on the interior of said reaction chamber having a first reaction component deposited thereon in solid form and in predetermined amount and at least one of said sections presenting an interior surface area of said auxiliary chamber having a second reaction component deposited thereon that is different from said first reaction component and that is in solid form and in predetermined amount.

19. A disposable reaction container according to claim **18** wherein said second component is a substrate enzymatically reactive with said first component and wherein said first and second reaction components are deposited on their respective surface areas by lyophilization in situ.

20. A disposable reaction container according to claim **15** wherein said auxiliary chamber comprises a primary zone and a buffer zone, said buffer zone being interposed between said primary zone and said opening of reduced cross-section, effecting substantial separation of said primary zone from said opening and having substantially greater length than width.

21. In a method for determining change in optical density resulting from a chemical reaction wherein the reaction is carried out in a reaction container provided with spaced transparent windows arranged for successive transfer of light therethrough and through a portion of the reaction mixture disposed therebetween and wherein components of the reaction mixture are dissolved in a solvent medium and the reaction is evidenced by the change in optical density following completion of the reaction mixture, the improvement which comprises placing at least one of the components of the reaction mixture in solid form in said reaction chamber, placing a key component in solid form essential for initiating the reaction in an auxiliary chamber of said reaction container that is adjacent said reaction chamber and is separated from said reaction chamber except for a passageway of restricted cross-section, introducing the solvent medium of the reaction mixture into said reaction chamber and thence into said auxiliary chamber in such manner that there is no substantial return flow of said solvent medium containing dissolved key component from said auxiliary chamber into said reaction chamber, thereby effecting solution of said solid components in said auxiliary chamber and in said reaction chamber in said solvent medium, introducing into said reaction chamber any other component required to complete the reaction mixture, and thereafter forcing solution containing said key component in dissolved condition from said auxiliary chamber through said passage into said reaction chamber thereby commingling it with the solution in the reaction chamber and initiating the reaction.

22. A method according to claim **21** wherein after the introduction of said solvent medium the reaction container and its contents is brought to a predetermined reaction temperature prior to forcing solution from said auxiliary chamber into said reaction chamber.

23. A method according to claim **21** wherein solution of said key component is forced from said auxiliary chamber into said reaction chamber by application of air pressure.

24. A method according to claim **21** wherein after introduction of the solution of the key component into the reaction chamber a portion of the resulting mixture is returned to the auxiliary chamber and forceful injection into the reaction chamber is repeated.

25. A method according to claim **21** wherein said key component in a substrate of an enzymatically catalyzed reaction with a component in said reaction chamber and wherein after the solvent medium has been introduced a material comprising the catalyzing enzyme is introduced into the reaction chamber prior to forceful injection of solution of the substrate into the reaction chamber.

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195—54; 23—230 B, 253

UNITED STATES PATENT OFFICE

CERTIFICATE OF CORRECTION

Patent No. 3,691,017 Dated September 12, 1972

Inventor(s) George H. Brown and Robert J. Ewing

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Col. 9, line 29, delete "chamber. Alternatively, a mechanical plunger could" and substitute --chamber may be relieved so that the liquid level may--;

line 36, delete "punger" and substitute --plunger--.

Col. 13, line 49, delete "relations" and substitute --relation--.

Col. 14, line 17, delete the period (.) and substitute a comma (,);

line 50, after "window" insert --in--.

Signed and sealed this 13th day of February 1973.

(SEAL)
Attest:

EDWARD M.FLETCHER,JR.
Attesting Officer

ROBERT GOTTSCHALK
Commissioner of Patents