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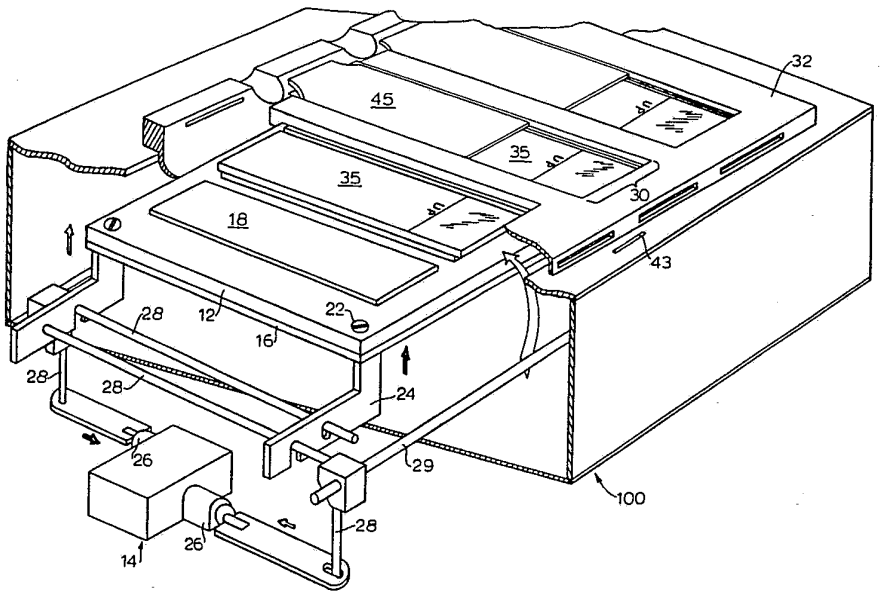

(57) Abstract

The invention is an apparatus and method for performing automated sample preparation, DNA amplification and detection, which includes specimen carriers (30) for holding specimens and reagents, device for heating (12) and cooling (48) and maintaining the specimen to or at any given temperature for any given time periods, and a computer (60) to generate signals that control temperatures and times. The specimen carrier (30) may be used with other kinds of heating (12) and cooling device (48) as an evaporation-controlled device when the specimen carrier base (33) and cover (44) are brought together to form a chamber over the biological specimen area (58).

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APPARATUS FOR CONTAINING AND THERMAL PROCESSING OF BIOLOGICAL SPECIMENS

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to the field of automated analyzers for nucleic acid diagnostics, in particular to temperature control devices which can quickly heat and cool, maintain a stable setpoint and contain the sample and reagents during thermal cycling.

Description of the Related Art

This invention more fully describes an embodiment of the carrier which incorporates a standard microscope slide as part of the carrier and a temperature control system and its integration with a carrier assembly for more precise temperature regulation, as first described in U.S. Patent No. 5,188,963 issued February 23, 1993, and also in international patent application No. PCT/US90/06768. The carrier assembly comprises multiple carriers, which may use a microscope slide as the main portion of each specimen carrier base. The carrier in the aforementioned references contains a fixed biological specimen during biochemical and molecular reactions and allows a first reagent to be added and displaced by a second reagent or wash through a unidirectional fluid-flow channel, said channel defined by the carrier base and a cover, through which reaction fluids are introduced for complexation with target molecules of a specimen, with the provision of a collection trough into which spent fluids drain from the carrier base. In this invention another embodiment of the carrier cover forms at least one closed chamber over a standard microscope slide or a carrier base during steps in the processing requiring evaporation control.

The *in situ* amplification process (described in U. S. Patent No. 5,188,963 and international patent application No. PCT/US90/06768 filed November 16, 1990) uses enzymes such as polymerase or ligase, separately or in combination, to repeatedly generate more copies of a target nucleic acid sequence within cells by primer extensions to incorporate new nucleotides or by ligations of adjacent complementary oligonucleotides, wherein each template generates more copies and the copies may themselves become template. By melting complementary strands of nucleic acids, the original strand and each new strand synthesized are potential templates for repeated primer annealing or ligation reactions to make and expand the number of specific, amplified products. A thermostable polymerase with reverse transcriptase activity and

a thermostable ligase are now both available and increase the choice of enzymes and combination of reactions for *in situ* applications. If RNA in the sample is the target to be amplified, the sample is treated with reverse transcriptase to make a nucleic acid complement of the RNA just prior to amplification. The amplification can either be primer extensions in one direction for linear amplification, or in opposing directions, for geometric amplification. The label can either be incorporated as labeled nucleotides or labeled primers for one-step detection or labeled probes may be added in a step following amplification whereby the probes hybridize to the amplified products for detection.

Until *in situ* amplification was invented, nucleic acid amplification was limited to solution reactions wherein the nucleic acid is released from cells or tissue prior to amplification of the target sequence. In the aforementioned patent and patent application, a process to amplify nucleic acid targets within cells and a method for immobilizing the cellular specimens during amplification and detection was invented. A photomicrograph of cells which had amplified, labeled DNA was included in patent application No. PCT/US90/06768 to show that the amplified fragments are retained in individual cells and such cells can be enumerated under microscopic observation.

The process requires at least one denaturing or high temperature stage, and one primer annealing or low temperature stage in each cycle. Since the specificity of binding one nucleic acid oligonucleotide or strand to a complementary nucleic acid is influenced by temperature, uniform and accurate temperature from sample to sample is needed for the reaction. The time required for the sample to be brought to the reaction temperatures can be a large percentage of the time allowed for the biochemical processes to be performed; therefore, means to cycle the temperature of small volumes of reagents rapidly and reliably are desirable.

There are various techniques and devices for adjusting temperature of reagents and specimens thereafter controlling the reaction temperature. For example, it is known to use individual reaction heating coils around individual reaction vessels. While a circulating air or water bath can control temperature of a large number of reactions simultaneously, the rate at which heat transfers from such a bath to a reaction vessel is substantially proportional to the difference between the temperature of the vessel and the temperature of the bath, to the heat capacity of the fluid, and to the efficiency of the contact therebetween.

The aforementioned *in situ* amplification for cellular analyses, which requires precise temperature regulation, creates a need for an improved apparatus which adjusts and controls the temperature of the cellular specimens. An apparatus designed for rapid temperature cycling necessitates reducing thermal loads to increase the rate at which heat transfers occur. The carriers used in this invention are thin, flat reaction vessels whose base transfers and spreads the heat quickly to the ultra-thin specimen within. The chamber has a thin configuration over the specimen holding area to minimize the volume of reagents needed to saturate a thin specimen. When glass slides are inserted in a carrier assembly, each glass slide becomes part of the heat flow transfer to and from a specimen. A specimen in the thin, aforementioned configuration has greater surface contact with the slide, thereby reflecting quicker temperature changes. A flat configuration of the specimen holding area on a specimen carrier enables convenient microscopic analysis of molecular targets within the individual cells.

A device which provides heating and cooling of sample and reagents without evaporative loss is useful in many processes and particularly useful in gene amplification and detection processes. In this invention covers are placed over the specimen areas of slides forming individual evaporation-proof chambers into which reagents are either placed before sealing to the slides or preferably filled after the covers are sealed to the slides. Said device is interchangeable with the fluid-flow cover as designed previously for sequential addition of a series of reagents and washes for detection. It is clear that the chamber covers may be used to enclose specimen areas on the upper surface of microscope slides to control evaporation with temperature-controlled means other than those described herein.

It is commonly known to seal or cement cover slips to slides in order to preserve a specimen. The current techniques to prevent evaporation of reagents at elevated temperatures consist of covering the specimen, such as cells or tissue fixed on a microscope slide, with a cover slip and sealing the cover slip with either rubber cement, finger nail polish or similar adhesives and/or overlaying the specimen with mineral oil as a vapor barrier. In the former case the adhesive bonds must be broken to remove the cover and in the latter case the mineral oil must be removed in order to continue further processing. These techniques are unsatisfactory because they are messy, labor-intensive and introduce unwanted material and additional steps.

Once a chamber is made with the slide or carrier base, means of adding reagents

within it are necessary. Means of adding solutions to closed systems include injection ports of self-sealing natural rubber. Applying reagents with a standard pipette tip or the like is more advantageous. Flexible tubing may be clamped or crimped to seal after pipetting reagents through the tubing. The device of the invention improves on all of these possibilities by sealing the chamber, providing simple means of introducing reagents into the chamber and reclosing it while also providing means to vent pressure.

The aforementioned *in situ* amplification for cellular analyses creates a need for an improved apparatus which controls the temperature of the cellular specimens and prevents evaporative loss during thermal cycling. The invention deals with increases in air and vapor pressure within a sealed chamber as air and water are heated. A device designed to make a reaction chamber over slide specimens such that the chamber is sealed sufficiently to prevent evaporative loss during thermal cycling, but which may be easily removed or converted to a chamber in which subsequent reagents may be added and washed away from the specimen is desirable.

SUMMARY OF THE INVENTION

The present invention overcomes the limitations and drawbacks described above and provides reaction chambers for specimens and means to rapidly bring specimens to a higher or lower predetermined reaction temperature, to deliver unique fluids to individual areas of the specimens, to control evaporation of fluids during heating and can be readily adapted for use in an automated analyzer of DNA diagnostics.

The object of the invention is to detect molecular targets in thin biological specimens and accurately control the temperature of the specimens during simultaneous biochemical reactions, bringing all the individual reactions to a desired temperature, holding the reactions to the specified temperature for a period of time, cooling the reactions to a desired temperature, and holding the reactions at the specified temperature for a period of time.

In accordance with the present invention, an apparatus for providing a controlled temperature environment for a plurality of specimens includes an assembly for receiving specimen carriers, a heating plate with means to raise and lower its position relative to the plane of the specimens and means to create a laminar air flow between the specimens and the heating plate to cool the heating plate and specimens rapidly. A heating element in thermal contact with the heating plate heats the heating plate therein to a predetermined reaction temperature. The base of the carrier assembly contains an

ultra-thin specimen on its upper surface, and a bottom surface contacting the heating means, enabling heat to transfer and spread quickly to or away from the specimen. The apparatus also includes sensing means in thermal contact with the heating plate for sensing the temperature of the apparatus and controlling the heater to reach and maintain the specimen at the predetermined temperature.

Means to control evaporation for a plurality of specimens consists of one or more covers with means to make a seal with the specimen area of a slide or carrier base. Individual chambers are formed by sealing each cover over all or a portion of the specimen area. Covers are preferably one-time use disposables and they could be formed in multiple units linking two or more together so that one molded unit provided separate chambers for different specimen holding areas.

In one embodiment disclosed herein, the apparatus is generally rectangular in shape with specimen carriers in rows and includes a plurality of platforms on the heating plate extending upwardly therefrom, all in thermal contact with the specimen carriers when the heating plate is in the raised position. When the heating plate is lowered, a plenum is coextensive with the space between the specimen carriers and the heating plate, providing means for a laminar air flow to quickly cool the specimens and heating plate. It is understood that other embodiments are equally feasible such that, for example, the specimens could be arranged annularly in an apparatus having an annular heating plate and carrier assembly. In yet another embodiment the heating plate and the carrier assembly may be arranged more vertically than horizontally so that a closed position and an open position (for the distance between the heating plate and carrier assembly) is more descriptive than a raised position or a lower position for either the heating plate or the carrier assembly. While the heating plate moves in the embodiment described herein and in Figures 1-6, it is equally possible that the heating plate is fixed and the carrier assembly moves either to contact the heating plate or create a space for the laminar air flow.

In the preferred specimen carriers, the reaction area is thin and flat wherein the biochemical reactions are performed in a thin aqueous film or matrix rather than in standard tube or cuvette-type containers. The thin, flat specimen carriers are best suited for *in situ* DNA amplifications and detections which integrate specimen collection, preparation and gene detection in one reaction vessel. In the instances where the specimen to be analyzed is put on a standard glass slide for the convenience of

microscopic observation, a carrier assembly holding the slides supplies carrier edges and top pieces, and said carrier assembly incorporates other features of a supporting carrier rack such as providing the collecting trough. The glass slide is inserted in the carrier assembly which is then placed in the apparatus for processing just as carriers are positioned in racks as described in patent application No. PCT/US90/06768.

To accomplish precise heating and cooling, the present invention utilizes a specimen carrier assembly with openings through which a surface of each specimen carrier is in communication with a heating plate. Heating elements, sandwiched within or beneath the heating plate, heat the heating plate and transfer heat quickly and uniformly to the specimen carriers. Means to move the heating plate away from the specimen carriers break communication between the specimen carriers and the heating plate, and cooling commences immediately. A fan directs a laminar air flow between the surface of the heating plate facing the carrier and the surface of the specimen carrier facing the heating plate. The laminar air flow serves as a medium for the transfer of heat away from both the heating plate and the specimen carriers for rapid and uniform cooling.

The laminar flow cooling system of the invention cools thin, flat specimen containers. Said containers could resemble thin-walled cuvettes or tubes having a thin specimen holding area. The difference which defines laminar cooling is that air between the specimen holders and the heat source is compressed into a rapidly moving stream to cool objects on both sides of the air flow quickly and representative temperatures of both the heating plate and the specimen containers are monitored and adjustments are made in the air flow rate to bring each toward the temperature of the other. The apparatus of this invention provides for control of the temperature of specimens in the carrier and control of the distance between the heating plate and the carrier and control of the laminar air flow cooling. The slides' specimen holding areas are aligned with the raised heating platforms. The distance between each slide and the corresponding heating platform is uniformly adjusted and may be changed during heating and cooling. Other specimen containers having a thin specimen holding area and made of thin pieces to transfer heat efficiently and which use a laminar air stream for rapid cooling, as described herein, are within the scope of this invention.

In one embodiment disclosed herein, the carrier base is generally rectangular in shape and the cover holder includes a plurality of pressure bars in a linear arrangement

with means of aligning the cover over the carrier positions and pressing the covers to the specimen areas wherein each cover overlays an individual specimen area and each cover-slide pair forms a sealed humidity chamber for thermal cycling. The chamber formed within the cover provides a thin space over the specimen in the specimen holding area whereby a small volume of reagents is spread over the surface area of the specimen area and retained during thermal cycling. The chamber is thin enough to be filled by reagents and shaped so that newly-formed bubbles migrate to the neck region and do not limit reagent availability to the specimen. Means of sealing include a machined or molded rim around the edge of the cover, and the said rim is placed in physical contact with the slide. Conformal or gap-filling coatings and adhesives which add resilience or stickiness, but which do not bond permanently to a slide or carrier base, may be included as means to seal the chamber. A variety of spring means may be included to apply whatever force is needed to make a watertight seal. The degree of force needed depends upon the materials of which the cover is made and/or applied at the cover rim. When the cover is sealed against the slide it provides a space extending from its inner surfaces which is coextensive with the space above the specimen, and said space becomes a humidity chamber.

It is understood that other embodiments are equally feasible such that, for example, the cover holder comprises covers arranged annularly for use in an apparatus having an annular heating plate and carrier assembly.

Further objects, features and advantages of the invention will become apparent from a consideration of the following description, taken in conjunction with the accompanying drawing figures.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a perspective view of the apparatus of the invention showing one embodiment of a heating plate and lifting mechanism and a cut away view of a carrier assembly holding standard microscope slides. Slides are positioned in the carrier assembly on three of the four raised platforms to show how said slides contact the heating plate. Two of the carrier positions are shown with fluid-flow covers.

Figure 2 is a perspective view of a carrier assembly holding four glass microscope slides with one of the four slides in a partially inserted position. A dotted line shows one of the covers going from an open to a closed position.

Figure 3 is an enlarged view of the apparatus showing the path of the laminar air flow between the carrier assembly and the platforms on the heating plate when a measured distance separates the heating plate from the carrier slide bottom.

Figure 4 is an enlarged view of the apparatus showing cessation of the laminar air flow path between the carrier assembly and the platforms on the heating plate when a minute distance separates the heating plate from the carrier slide bottom.

Figure 5 is a partial, cross-sectional view of a carrier assemby and a heating plate taken along line 5-5 in Figure 3 showing individual raised heating platforms on the heating plate in alignment with slides positioned in the carrier, and corresponding fluid-flow covers over the individual specimen holding areas.

Figure 6 is a perspective view of a the cover holder which closes over the carrier with its pressure bars in alignment with the carrier positions. Also shown is a cover actuator used to close the carrier covers over the specimen holding area.

Figure 7 is a general block diagram of the temperature cycling apparatus.

Figure 8 is a logic flow diagram to show the steps in temperature control.

Figure 9 is an enlarged view of a modification of the cover holder in Figure 6 to position another embodiment of a cover. Slots in the cover holder's pressure bars are shown with a chamber cover positioned between the pressure bars.

Figure 10 is a cross-sectional view of a chamber cover taken along line 10-10 in Figure 9, similar to the cross-sectional view in Figure 5, but showing a chamber cover replacing a fluid-flow cover.

Figure 11 is a diagrammatic sketch of a cover design integrating fluid-flow and a chamber into one cover. This design is capable of forming three chambers and a separate fluid-flow channel leading to and away from each of the three chambers.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS THEREOF

The invention broadly comprises an apparatus for heating and cooling multiple specimens within carriers or microscope slides in a carrier assembly with covers temporarily placed over a desired specimen area for processing. The specimens are thin for cellular analysis and allow for rapid temperature change in the carrier assembly. With reference to Figures 1-11, a temperature control apparatus 100 in accordance with the present invention includes a heating plate 12, a lifting mechanism 14, heating element 16 and a carrier assembly 32. The heating plate 12 is preferably formed of a

heat conductive material such as aluminum alloy or copper. Heating plate designs were referred to in patent application No. PCT/US90/06768. The heating plate surfaces closest to the carrier assembly 32 may have protruding sections in a pattern that permits intimate contact with the carrier bottoms and specific means of heating, such as insulated resistive heating wire elements, may be incorporated or disposed in specific locations within the heating plate 12 by milling cavities in the heating plate 12 to direct heat to specific areas of the carrier assembly 32. Heating elements are fixed within such cavities by means known in art, for example, laser welding, to enclose the heaters.

The heating plate 12 embodied herein has raised platforms 18 integrally formed with the heating plate 12, for example, by machining or die-cast injection molding, or the platforms may be separately formed and bonded to the heating plate by soldering, brazing or with a suitable heat-conductive epoxy compound. If the platforms 18 are formed separately with separate heating elements positioned with the individual platforms 18, the heating plate 12 may be formed of aluminum, or an aluminum frame with as little thermal mass as possible, and the platforms 18 formed from copper. In all cases the heating plate surface meeting the carrier assembly 32 must be shaped so that intimate contact is achieved overall for optimal distribution of heat. In the preferred embodiment disclosed herein, the heating element is an insulated thermofoil material (Minco Products, Inc., Minneapolis, MN) having a total resistance in the range of about 6-16 ohms and being adapted to dissipate approximately 12 watts of power per square inch when 24 volts DC is applied thereto.

In the preferred embodiment disclosed herein, the temperature sensors 20A and 20B comprise thermistors, or thermocouples, bonded to, or embedded in, the heating plate 12 and a position thermally rate-matched to representative carrier, respectively. The temperature sensors 20 may have a nominal resistance of approximately 10,000 ohms at 25° C. Electrical connections for both the heating elements 16 and temperature sensors 20 are provided by means of feed-throughs. The heating plate 12 has screws 22 and posts 24 connecting it to the lifting mechanism 14. The posts 24 are preferably made of non heat-conductive material and are attached to the heating plate with non-metal screws 22. The lifting mechanism consists of means to raise and lower the heating plate and may be accomplished by any number of possible assemblies such as a combination of squeeze clamp solenoids and levers, or an electric gear motor and cam action. A further lifting mechanism may comprise a stepper motor, switch and double helix, whereby the heating plate is raised and lowered by moving one centrally-located

post up and down. Four arms from the center post to each corner of the heating plate support the heating plate, and movement of the double helix raises and lowers the center post. Spring-like action, executed from below the heating plate by means known in the art such as gaskets between the arms and the heating plate "float" the heating plate so that the heating plate surface is aligned with respect to the carrier assembly 32 when the heating plate is raised.

In the preferred embodiment of the lifting mechanism disclosed herein, four tubular solenoids 26 move four lever 28 arms at the same time to lift the four corners of the heating plate 12 and position its top surface with raised platforms 18 in immediate contact with the specimen carriers 30. Referring to Figure 1, two pairs of arrows show the direction each squeeze clamp moves in order to lift the opposite corner of the heating plate. One of the open arrows shows the direction one solenoid clamp 26 retracts to lift the opposite corner of the heating plate in the direction indicated by the other open arrow when lever arms 28 rotate around a pivot rod 29. The stippled pair of arrows demonstrates a similar action for the other solenoid clamp and heating plate corner.

With continued reference to Figures 1 and 2, the carrier assembly 32 is composed of specimen carriers 30 containing the material to be assayed which may be solid or liquid tissue specimens embedded or immobilized on the carrier bottom piece 33. The microscope slide 35 or specimen carrier 30 may be precoated with compounds such as aminosilane known in the art to adhere specimens to slide surfaces. In the embodiment shown the carrier assembly 32 has a plurality of rectangular openings 34 formed therethrough adapted to receive standard microscope slides, each of which said microscope slide 35 becomes part of the carrier base 33. The openings 34 are aligned with respect to the platforms 18 on the heating plate 12 so that each specimen carrier bottom 33 or microscope slide 35 makes thermal contact with the respective platform 18 when the heating plate 12 is in the raised position.

Referring to Figs. 5 and 6, a cover holder 38 is comprised of pressure bars 40 and the pressure bars 40 have slots 41 which help situate carrier covers. The cover holder 38 is fastened by means of hinges 42 to the apparatus housing and presses against the specimen carriers 30 between the slide openings 34 when said cover holder 38 is closed by a spring-loaded closure 43, insuring thermal contact when the heating plate 12 is in the raised position. One of two types of covers is used to cover a specimen carrier 30. One type of cover is a chamber cover 44 and another type of

cover is a fluid-flow cover 45. In the embodiment shown in Figs. 5 and 6 the cover holder 38 has a cover actuator 46 which holds or grips the covers. The cover actuator 46 grippingly moves over the pressure bars 40 to apply pressure to fluid-flow covers 45 or chamber covers 44.

With further reference to Figure 3, a fan 48 connects to a plenum 50 coextensive with the laminar air flow space 52, created when the heating plate 12 is retracted a distance of 2-10 millimeters from the carrier. A baffle (not shown) made as known in the art is configured in such a way within the plenum 50 so as to even out the rate of air flow entering the laminar air flow space to all specimen carriers 30. The carrier's base 33, or a microscope slide 35 which is inserted as part of the specimen carrier 30, form the upper boundary of the laminar flow space 49 and the heating plate forms the lower boundary of the laminar flow space 49.

In Figure 4 the distance is reduced between the heating plate and carrier to show the heating plate touching the carrier bottom piece. As disclosed in patent application No. PCT/US90/06768, actual temperature data representing the heating plate and the carrier were recorded using a prototype device. The carrier held 25 mm X 75 mm glass slides and the distance between said heating plate and slide was constant during cycling. The importance of the data is that heat convected from the heating plate via the air cushion overcame differences in starting temperatures at different cycles to bring the slide closer to the desired higher temperature setpoint, but lower temperature setpoints varied from one cycle to the next. The data suggest that adjusting the distance between the heating plate and the carrier is as least as important, or even more important, in maintaining consistent lower setpoint temperatures versus higher ones. The data also demonstrate that temperature cycling control is possible without intimate contact between the heating plate and the carrier with each in a fixed position, the fixed position affects the slope of the heating/cooling curve, and preferably the distance (which may be 0-2 cm, or somewhere in between at a particular point in the temperature cycling) changes during the programmed temperature cycle. The instant invention improves temperature regulation by controlling a laminar air flow between the heating plate and the carrier. The invention further provides mechanisms and computer means to control changing the distance between heating plate and carrier. Referring to Figure 4, the elevational view of one embodiment of a lifting mechanism 14 further shows the positional change of the solenoid-operated 26 lever arms 28 as the lever arms rotate to lift the heating plate 12 at each of the four posts 24. The dotted

line shows where the heating plate meets a slide 35 within the carrier assembly 32. Arrows show the direction of rotational movement of the lever arms around the pivot rod 29 to lift the heating plate 12.

The cross-section view of the carrier assembly 32 in Figure 5 is located in Figure 3 by the line marked 5-5. The heating plate 12 is shown in a retracted position relative to the carrier, as in Figure 3, to illustrate the laminar air flow space 52. The platforms 18 on the heating plate 12 are shown aligned with the slide 35 and carrier bottom 33.

The preferred thickness of the carrier bottom is 1 millimeter or less. Figures 5 and 10 are not drawn porportional in order that the thin structures show up better on the drawings. The carrier assembly 32 is made of a heat-resistant material and may formed as one plastic piece by compression or injection molding processes to hold slides 35. Alternatively, the carrier assembly 32 may be made by arranging separate sections, which sections are cut from long extruded plastic into appropriate lengths, whose cross-section is shown as extrusion piece 54 in Figure 5, and which are placed at intervals to accommodates the slides and joined with cross pieces by means known in the art such as laser welding.

The cross-section view in Figure 5 also illustrates the sequence of parts through the carrier assembly and heating plate starting with the cover actuator 46, pressure bars 40, the carrier covers 45 in a closed position, the position of carrier edges 56 defining the height of the specimen holding area 58, the slide 35, carrier bottom 33, the laminar air flow spaces 52, the heating platforms 18 elevated above the main part of the heating plate 12, the heating plate 12 in a retracted position and the heater 16.

With further reference to Fig. 5, wherein individual, standard 25 millimeter by 75 millimeter microscope slides 35 are positioned under the carrier edges 56 and become an integral part of the carrier base 33, openings in the carrier base 33 are provided through which openings, sections of a heating platen permit intimate contact with the bottom of the slide 35. The specimen holding area 58 is an area on the upper surface of a slide 35, or specimen carrier 30, wherein individual specimens are placed. It is clear to see that the specimen carriers 30 may be formed either as one structure with separate specimen holding areas 58 or as separate pieces.

In the embodiment of the carrier disclosed in Fig. 2 herein, two fluid-flow covers are shown corresponding to the two rear specimen carrier 30. The type of cover is changed to accommodate one of two desired functions. A fluid-flow cover 45 is used

for sequential, temperature-controlled reagent and wash treatments and incubations. A chamber cover 4 is used when an evaporation-proof chamber is required for thermal cycling. The same specimen may be processed with both types of covers, but covers of the same type would likely be used together for the same steps of a protocol.

The cover holder 38 has a cover actuator 46 which raises and lowers covers 45 when applied force slides it forward and back over the pressure bars 40 (Fig. 6). The same pressure bars 40 may be used to exert spring tension between the cover holder 38 shown in Fig. 6 and chamber covers 44 sealing each of them over the specimen holding areas 58 and making individual, evaporation-proof chambers.

In Fig. 9 a portion of a cover holder 38 is shown to illustrate how a chamber cover 44 is held in slots 41 in two opposing pressure bars 40. In this embodiment the chamber cover 44 comprises a molded piece incorporating two wing tabs 47 so that when wing tabs on opposite sides of the chamber cover 44 are inserted into matching slots 41 in opposite pressure bars 40 and a force is applied to the cover holder 38 and pressure bars 40, the resulting spring tension presses the chamber cover 44 against the specimen area of a slide 35 or carrier base 33. The actuator could also be configured as a means to apply force to chamber covers 44 (not shown). Other embodiments may include leafsprings appropriately positioned in the cover holder or a bayonet and socket connection between the cover holder and the chamber covers. Bayonet rods protruding radially from the sides of a chamber cover would follow the tracks in a bayonet socket in a cover holder and allow the chamber cover to be locked into the cover holder with a push and a twist.

In reference to Figs. 9 and 10, a chamber cover 44 comprises the wing tabs 47, which are extensions of a step 49, a neck 51 and a flange 53. A chamber cover 44 secured over the specimen holding area 58 forms a chamber such that a space over the specimen is coextensive with a space under the cover. The step 49 portion and any extensions of it, such as the wing tabs 47, bear the load of the cover holder 38 pressing against it, and distribute the force to the flange 53. The neck 51 is a generally vertical portion of the chamber comprising a central cylindrical channel 55 and a plug 57 to close the channel 55. The flange 53 comprises the portion of the chamber cover 44 extending from the step 49 which makes contact with a specimen area on the slide 35 or carrier base 33. The rim of the flange 53 is machined or molded to seal against the slide 35 or carrier base 33. Other means to enhance sealing include gaskets, conformal coatings or adhesives which may be added to fill gaps and/or add resilience to the rim

of the flange 53. Since the chamber cover 44 is removable so the specimen may be further analyzed, the means of enhancing sealing should be temporary and coatings or adhesives are selected on the basis of characteristics making them easy to apply and adhere to the cover, but which do not bond the cover permanently to the slide 35, or carrier base 33. Examples of conformal coatings are one component, self-leveling silicones (Shin-Etsu Silicones of America, Torrence, CA). Examples of gaskets may be O-rings with cross-sections of preferably 1 millimeter, or less, which are embedded in the flange 53, or flat disc-like washers made of resilient material, placed to make a seal between the rim of the flange 53 and the specimen holding area 58.

A circular chamber with a diameter of 0.40 inch at the specimen area, for example, would be made by positioning a chamber cover 44 of the same internal diameter over the specimen area 58. The outer diameter of the chamber cover 44 is a slightly larger diameter to take into account a rim thickness in the range of .010 - .030 of an inch wide. Preferably the maximum height of the chamber is 0.020 inch and slopes to a minimum height of 0.005 inch at the cover rim. A chamber cover 44 of said dimensions defines a chamber with a maximum holding capacity of approximately 35 microliters including filling the lower portion of the channel 55 in the neck 51. The chamber is designed to utilize small reagent volumes efficiently. Dimensions of the neck 51 are large enough to comprise a cylindrical channel having diameter of at least 0.1 inch and preferably a Luer taper having a diameter which decreases 6% from 0.176 inch to 0.152 inch from the top to the bottom of the channel 55. The outer diameter of the neck 51 is of sufficient width to have a channel 55 within of at least 0.1 inch diameter. The neck 51 and its channel 55 are tall enough to accommodate expanding and contracting liquid volumes during thermal cycling. The diameters of the neck 51 and channel 55 remain relatively constant, although the dimensions of the step 49 and flange 53 may be smaller or larger to accommodate a smaller or larger specimen holding area 58 on the slide 35 or carrier base 33.

The covers are made of a heat-resistant plastic material such as polypropylene, polycarbonate, polysulfone, polyetheretherketone or teflon and blends, welds, coatings or laminations of these and other appropriate materials. Polyetheretherketone has excellent chemical and heat-resistance and has been used to machine reusable covers which were successfully tested for use in *in situ* DNA amplification. The surface and curing properties of materials are a significant consideration in selection because the materials need to be free of compounds or surface charges acting to bind reactants or

inhibit enzymatic reactions or interfere with detection and interpretation of test results.

Reagents are added to the chamber through the channel 55 in the neck 51 after the chamber cover 44 is sealed against the specimen holding area 58. The minimum channel girth was experimentally determined for convenient addition or removal of reagents. Liquid reagents are easily added to the chamber through a channel 55 of at least 0.1 inch diameter by inserting a standard pipette tip or liquid delivery probe into the channel 55. Air within the chamber escapes around the pipette tip as the liquid reagents displaces it. Alternately, reagents in a dry formulation or in an agarose gel matrix may be placed in the chamber before the chamber is sealed over the specimen.

Chamber ceilings were sloped at a 3° angle to fill without trapping bubbles. The chamber height is similar to capillary space that draws the liquid into it. If a small bubble does form in chambers having a wider diameter, the cover can be pressed gently and burped before plugging the channel. An objective of the chamber design is such that not only the first air in the chamber and gases dissolved in the liquid reagents, but also that air collecting in the chamber during thermal cycling escapes into the channel 55. Because the chamber ceiling slopes up to the neck 51, the neck channel 55 becomes an air space above the level of reagents in the chamber and one in which bubbles collect. It is important that air bubbles are not present in the main part of the chamber which may restrict accessibility of the reagents in the specimen holding area 58 or which may partition the aqueous reagents into two parts, one containing increasing concentrations of solutes over the specimen holding area 58 because the other part consists of water droplets condensing on the ceiling. If partitioning were to occur because newly-formed water droplets formed and did not return to mix with the initial solution, the change in molarity of solutes of the solution bathing the cells may affect biochemical and enzymatic activity. Sloping the ceiling and keeping it close to the specimen holding area 58 force the air bubbles into the channel 55.

The plug 57 needs to be secured in the channel 55 to prevent leaking and in our experience a Luer taper fit for the plug in the channel worked best, provided that the plug 57 and chamber cover 44 materials were compatible. Since the diameter of the channel 55 is just sufficient to add reagents, regardless of the cover rim diameter, the surface area of the plug 57 upon which condensation may occur is much less than the surface area of the chamber ceiling. It is desirable that the surface of the channel 55 and the plug 57 have a hydrophobic character so that condensation beads up more readily, causing water droplets to drip back into the initial solution, thereby reducing

partitioning and its consequences.

If the neck 51 and plug 57 were heated from above, it is clear that less condensation would occur on their surfaces and refluence of water vapor in the chamber would be reduced. However, in the device of the invention the portion of the chamber where air bubbles collect is limited to the channel 55 in the neck 51. Confining refluence to the channel 55 reduces the effect it has in the main part of the chamber over the specimen holding area 58. Studies with cover designs in which air bubbles collected in the specimen holding area 58 resulted in cells in areas of greater reflux being more stained, thereby affecting uniformity of results. The objective of the chamber design is to confine the area of refluence and provide a uniform refluence, if it is not eliminated altogether. Filling the generally horizontal main portion of the chamber with reagents and limiting air space to the generally vertical portion in the channel 55 help to achieve these objectives.

For example, a chamber cover 44 of 0.4 inch diameter was filled with a volume of reagents, preferably in the range of 25-40 microliters, and plugged so that as little air as possible remains in the chamber. When heated over the temperature ranges required for sample processing, gases in an air space expand in volume more than three-fold and increase vapor pressure in the chamber. Pressure of aqueous vapor over water increases from 17.5 millimeters of mercury (mm Hg) at 20 ° C. to 760 mm Hg at 100° C. (Handbook of Chemistry and Physics, Sixty-third Edition, page D197, CRC Press, Inc., Boca Raton, FL). If the pressure of the confined water vapor that has accumulated above its liquid is rapidly increased by heating, the pressure increase of each thermal cycle puts strain on the seal made between the cover rim and the specimen holding area 58 or the seal made between the channel 55 and the plug 57. The pressure was vented to put less strain on the seals and require less pressure to be applied to the chamber cover 44 from the pressure bars 40. Venting was accomplished with means that prevented vapor from escaping by incorporating a flexible portion or bladder 59 in the chamber. As the chamber contents is heated and pressure builds up, the bladder 59 expands in volume and bulges outward from the chamber defining a second, larger volume.

The size and shape of the bladder 59 is selected to compensate for the increased volume of reagent when it is heated. The volume changes which were measured ranged from 15-30% and were affected by how much air was within the chamber after the liquids were added and by how much air was dissolved in the liquids initially. The

bladder 59 may be placed within a plug 57 having a hollow core and containing an elastic material such as a silicone gel. A simple kind of bladder 59 used was a thin film with silicone/rubber blended adhesive that stuck to the top of the neck 51 and worked well both as pressure relief and a vapor barrier.

Other materials used to relieve pressure and prevent evaporation were mineral oil (Aldridge Chemical, Milwaukee, WI), wax pellets (Perkin Elmer, Norwalk, CT) and a liquid wax (MJ Research, Inc., Watertown, MA). The capability to prevent evaporation was increased using mineral oil or wax as plugs when combined with an adhesive film cover. The mineral oil is a less desirable means because it is difficult to remove entirely from the specimen and affects staining. Cooling the chamber, before removing the chamber cover 44, solidifies the wax in the channel 55 so that when the chamber cover 44 is removed, the wax is also physically removed from specimen holding area 58.

Alternately, venting the chamber may also be accomplished by incorporating a membranous material into the channel so that gases, except for water vapor, move in and out. A hydrophobic, extra-fine pore size, filter material #7751 (Porex, Philadelphia, PA) was tried and determined to be more useful when it was compressed, but not totally effective, in preventing the escape of water vapor at the temperature and pressures achieved in the chamber. If a filter material meeting the specifications of thermal cycling in the chamber is, or becomes available, it can replace adhesive films, mineral oil, waxes, and/or silicone gels.

Using Luer-tapered openings in the chambers is a way to secure a tight fit for plugs made to contain thin elastic films, gels, liquid waxes, and other materials which are easily pierced with automatic fluid probes. Another embodiment of the device of the invention is a cover holder molded with multiple covers which are aligned and joined in manufacturing to the corresponding 96 wells of a standard microplate. In the aforementioned device, channels in the neck of each of the microplate's 96 wells, or chambers, comprises a Luer tapered channel. The plugs fitting these Luer tapered channels may also be molded with a support that aligns the plugs in 96-well array. It is our experience that silicone gels such as two-part RTV's, 6166A/B and 6196A/B, (General Electric Silicones, Waterford, NY), mixed 1:1, could be dripped into male Luer connections which had been cut off the end of standard syringes. The gel will not flow through the Luer connection but cures within it. After curing the silicone, Luer connections placed in Luer-tapered channels provide a vapor barrier that can be

penetrated for automated fluid exchanges. The usefulness of the device of the invention is that the vapor barrier material may be placed in the plugs as part of the manufacturing process. A microplate format with 96 multiple specimen chambers joined to covers comprising corresponding 96 Luer-tapered openings are therefore capable of being sealed snugly with a single 96-multi-plug piece for thermal cycling. Advantages include reducing evaporation, no carryover of material from one of the well chambers to another and recovery of products from each reaction without removing the covers or plugs.

The carrier base 33, or slides 35, and cover holder 38 may be closed together by a spring-loaded closure before placing them in a temperature apparatus. Another embodiment of a cover holder 38 may have the means to grip or snap directly onto an individual microscope slide 35. Also within the scope of the invention are elliptical chamber covers or other shapes accommodating larger tissue specimens, multiple chambers per slide accommodating two or more specimens on the same slide, or adding different reagent sets to duplicate specimen samples on the same slide.

Another embodiment of a cover is a configuration forming channels leading to and away from individual chambers when the cover is pressed against a slide or carrier base. This embodiment illustrates a way to integrate the carrier described in U. S. patent No. 5,188,963 with the device of this invention. In our research, several designs were outlined with adhesive on different slides and after placing covers over the outlines, each design was tested for uniform flow to three specimen areas on one slide through three separate channels. The design selected by its capability for fluids to flow through best without air blocks is illustrated in Fig. 11. It is understood that various cover configurations may be made to match the desired surface area of the specimen holding area to-be-included in the chamber and such chambers would also have means for sealing and venting for thermal cycling. Along with this embodiment is one in which the neck of the cover extends away from the plane of the specimen at an angle, rather than vertical as shown in Figs. 9 and 10, but the fluid level in neck's channel nevertheless remains above the fluid level in the chamber.

Returning to Fig. 7, the apparatus is controlled by a microcomputer or CPU (microprocessor) 60 with memory 62. The user enters a heating/cooling profile into the computer via a keyboard 64 or touch pad in response to queries on the menu display 66. A profile comprises a time to heat to setpoint temperature SP_h (ramp), time T_h to reside at setpoint temperature (soak), a selected time to decrease temperature to a lower

setpoint temperature SP_l (ramp) and time T_l to reside at lower setpoint temperature (soak). Generally two or three different soak temperatures are selected by the user and default ramp rates are preset, but may be overridden if ramp time is also designated by the user. A temperature, SP_h, is preferably within the range of from about 60° C to 95° C. A temperature, SP_l, is preferably within the range of from about 35° C to 60° C.

The CPU programs comprise instructions to enter and store user profiles and interfaces with a temperature control circuit 68 which contains programming for the lifting control 70, heating control 72 and fan, or laminar air flow, control 74 as diagramed in Figure 7. The temperature control circuit 68 contains a proportional or a proportional-integral-derivative (PID) algorithm for heating and cooling control. Proportioning may be accomplished either by varying the ratio of "on" time to "off" time, or, preferably with proportional analog outputs as known in the art which decrease the average power being supplied either to the heater or the fan as the temperature approaches setpoint. PID control combines the proportional mode with an automatic reset function (integrating the deviation signal with respect to time) and rate action (summing the integral and deviation signal to shift the proportional band). The 1990/91 Temperature Handbook by Omega Engineering, Inc. (Stamford, CT) contains explanations of the various control modes in the "Introduction to Temperature Controllers" on pages P-5 to P-10. Such microprocessor control systems are well known in the art and need not be further described herein. Control functions required for automatic temperature control particular to the apparatus of the invention are more fully explained herein for each step in the logic flow diagram in Figure 8.

The process starts with a command to the CPU 60 from the user to begin temperature control in Step 82 of Figure 8. A user-defined temperature profile is selected from the computer's memory or entered from the keyboard to begin operation. After checking that the spring-loaded closure 43 is in a closed position, the heating plate moves to make physical contact with the slides. The lifting control 70 in this embodiment activates four solenoid-operated lever arms, SOL-1, SOL-2, SOL-3 and SOL-4 in Figure 7 to position the heating plate in contact with the carrier bottom 33.

The CPU monitors the temperature of the heating plate and, upon receiving the run command, issues the proper command signal to begin heating in Step 84. Upon receiving the proper command, the CPU retrieves the first setpoint data and issues a proper signal to cause heating for a user-defined temperature profile at a default rate and starts the clock. The heater heats the heating plate to the high temperature equal

to a user-defined level, which is referred to as temperature variable SP_h. The heater begins heating at full voltage and heats to the desired setpoint in the shortest time possible unless the user defines the time period for reaching setpoint temperature. Heat transfers from the heating plate by conduction to the carriers until the desired carrier temperature is reached and during the incubation period to maintain the temperature. The lifting mechanism remains activated until a set point temperature is retrieved that is lower than the previous one.

In Step 86 the CPU reads the temperature of heating plate as the temperature sensor 20-A (Figure 7) develops a signal as known in the art that is proportional to the temperature of the heating plate 12 and such a signal is converted to a signal for the digital temperature control circuit 68. The CPU monitors the temperature of the heating plate and issues the proper command signal to cause the heater to heat the heating plate until the desired temperature is reached, and then issues the proper commands to the temperature control apparatus to cause the desired temperature to be maintained. Using either the proportional or the proportional-integral-derivative (PID) algorithm, the CPU computes a set point as a target temperature, continuously monitors the temperature of the plate and compares it as it approaches the set point on the user-defined temperature profile. An error signal is generated by comparison of the actual temperature to the calculated set points in the algorithm. The temperature control circuit 68 generates a signal that is proportional to the error voltage applied thereto and the rate of change of such error voltage. The resulting signal from the temperature control circuit 68 generates a modulated output proportional to the signal applied thereto. The output is in turn applied to the heating element 16. The voltage to the heater is controlled by the temperature control circuit and may be turned on and off and the rate of heating may be tuned by adjusting the voltage.

When a slide 35, which has a temperature lower than the selected reaction temperature, is added to the carrier assembly 32, or, when a specimen carrier 30 that is already installed in the carrier assembly 32, is filled or washed with a fluid that is lower than the temperature of the heating plate 12, heat from the heating plate 12 flows to the specimen carrier 30 through the thermally conductive platform 18. In response to the heat flow, localized cooling of the platform 18 in the immediate area of the specimen carrier 30 draws heat from the heating plate 12. As this process continues, the temperature control circuit 68 with the temperature sensor 20-A and the heating element 16 operate as described above to maintain the reaction temperature of the

heating plate 12 at the predetermined temperature. Heat flow in the opposite direction occurs if the carrier assembly 32 has a temperature higher than the heating plate, and said heat is absorbed by the larger thermal mass of the heating plate, such that adjustments are made in the heater control.

In Step 88 the CPU keeps track of the elapsed time at particular temperatures to implement desired incubation periods. At least one temperature sensor 20-B (Figure 7) is placed in a specimen carrier 30 and used to develop a signal. The temperature is monitored via sensor 20-B and the CPU determines whether the carrier is at the correct process temperature. During ramp periods the carrier temperature may lag behind in ramping to a higher setpoint temperature or said carrier temperature may move ahead in ramping to a lower temperature at any given moment in time. For this reason it is important that elapsed time for incubation start when the carrier bottom, not the heating plate, attain soak temperature. The microcomputer control system may start counting the incubation period when temperature sensor 20-B, representing the temperature of the slide 35 or carrier bottoms 33, reaches the predetermined temperature. To implement timing of the incubation period, the computer restarts a clock and times the elapsed time from when the temperature sensor 20-B equals the temperature, SP_h. The incubation time variable is generally set by the user according to the requirements of a desired biochemical process.

In Step 90 the CPU compares the elapsed time that the slides are at the desired incubation temperature, SP_h, with the selected incubation time, T_h. If the actual time is less than the selected time, the program continues to maintain temperature and compare the elapsed time.

When the elapsed time that the slides are at temperature, SP_h, equals the desired incubation time as determined in Step 90, the CPU sends the proper command in Step 92 to the heating and cooling apparatus to cause the heating plate to be cooled toward a low temperature, SP_l, set by the user.

In some profiles the next setpoint temperature after moving to a higher temperature may be an even higher temperature. In this case the program reenters at Step 84. Control of laminar flow cooling is integrated into the temperature control circuit 68 as follows. When the next desired temperature of the heating plate 12 is lower than the present heating plate temperature, the temperature control circuit 68 develops a signal to the lifting control 70 to deactivate the solenoids, causing retraction of the heating plate 12. A simultaneous signal to the air flow control 74 activates the

fan 48. Air enters the fan 48 and is pressurized into the plenum 50 into a laminar air flow through the laminar air flow space 52. Air flows through the laminar air flow space 52 between the platforms 18 and specimen carrier bottoms 33, removing heat from the specimen carriers 30 and the heating plate 12. The laminar air flow space 52 is thin enough and the air flow pressurized enough by compressing it into a thin space that air turbulence is kept to a minimum.

The program returns to the proportional or PID algorithm in Step 86 to execute heating/cooling control towards a lower setpoint in a similar way in which control was executed towards a higher setpoint, but involving different output control signals. The transmission of commands by the CPU activate a laminar air flow to cool the heating plate and the carriers simultaneously. The temperature of the heating plate 12 and the slide 35 or carrier bottom 33 are monitored by the CPU and an error signal is generated by comparison of the actual temperature to the calculated set points in the proportional or the proportional-integral-derivative algorithm to control the temperature of the heating plate. Periodically, an error signal based upon the comparison between the computed slope of the user-defined temperature profile and that of the new set point is generated from calculation of the slope and the elapsed time. The error signal is converted to the proper control signal to control the lowering of temperature to a lower setpoint.

The speed of the fan is controlled by inputs from temperature sensors 20A (representation of heating plate temperature) and 20B (representation of slide carrier temperature) to the proportional controlling algorithm. Changing the speed of the fan increases or decreases the airflow so that the rate of cooling is within bounds of the user-defined time or the default rate set by the program. The adjustments in airflow compensate for fluctuations in the temperature of intake air and internal heat build-up within the apparatus. When sensor 20-B reaches the lower setpoint temperature, the clock starts counting the elapsed time set for the incubation period, T_I. If the error calculated by the CPU between sensor 20-A and sensor 20-B indicates that sensor 20-A is lower than sensor 20-B when the setpoint temperature is reached, the heater is activated; if sensor 20-A is higher than sensor 20-B, the airflow continues to cool the plate after sensor 20-B reaches the low setpoint temperature. Comparing thermal loss rates detected by sensors 20-A and 20-B during the cooling phase and making the aforementioned adjustments work toward equilibrating the temperature of the heating plate and the carrier just as the lower setpoint temperature is reached. At the point

when the heating plate and slide carriers are both very near the low setpoint temperature, a control signal activates the lifting mechanism to restore contact between the heating plate and the carrier and another signal to the fan control deactivates the fan. Maintaining a stable temperature for an incubation time period operates similarly for high and low setpoint temperatures. Heat loss to the surrounding environment requires activating the heater control to keep heating plate at low setpoint temperature.

The controller algorithm is also programmed to change fan speed when a differential temperature between sensor 20-A and 20-B is greater than a predetermined amount. Ideally, the heating plate and the carrier are designed to have balanced thermal load and heat loss characteristics. Variable airflow works to fine tune cooling so that when cooling is achieved in less than the user-defined ramp time, or a predetermined default time, or the temperature differential between sensors 20-A and 20-B is greater than a predetermined amount, a decrease in airflow allows more efficient convective transfer of heat from the heating plate through the air cushion to the carrier, or vice versa, and works toward achieving thermal equilibration before low setpoint is reached.

Step 88 following a cooling phase toward a lower setpoint temperature is the same as one that follows moving to a higher setpoint temperature. The CPU measures the elapsed time from the time the slides temperature reaches the SP_l of the heating plate.

Step 90 again compares elapsed time to the user-defined low temperature incubation time, T_l. As soon as the elapsed time equals the desired low-temperature incubation time, T_l, Step 92 involves the CPU retrieving the next setpoint temperature in the profile and continues until all setpoint temperatures have been executed.

When one profile is complete, the CPU counts the number of times the profile has been run and compares the number to a user-defined variable in memory. The number of times a profile is to be run is the cycle count. In Step 94 the CPU compares the cycle count to the user set variable. If the cycle count does not match the desired number of cycles, processing returns to Step 84. If the cycle count equals the set variable for the desired number of cycles, processing proceeds to Step 96.

After the desired number of cycles has been performed, Step 96 determines whether the user wishes to run another temperature profile stored in another "file" or database. Every temperature profile entered by the user has a link data field in which there is stored the profile identification of the next file or temperature profile to be run, if any. The contents of this field are read. If the field finds a profile number in the

link field, then processing returns to Step 84 and restarts by retrieving the first setpoint temperature in the new profile and continues processing through Step 96 again to achieve each setpoint temperature in the profile for the set number of cycles.

In Step 98 the contents of the data link field are read and if the user has made no further entry to the link field, signals to lifter and fan controls work to cool the heating plate until no further reduction in temperature occurs. When the heating plate temperature as sensed by temperature sensor 20-A is not lowered for a preset time period, an "end" message is displayed and the control functions shut off the temperature control apparatus.

The use of DNA amplification cycling temperatures for annealing and denaturation are both above ambient air temperatures, making refrigeration or Peltier-cooling of the specimen carriers unnecessary in automated clinical DNA analyzers. However, a means of refrigerating or Peltier-cooling air may be employed to prechill the air entering the fan and plenum, thereby augmenting the speed of cooling by increasing the temperature differential between the specimens and the air used to cool them.

A possible regime to process cellular specimens on standard microscope slides is as follows. The slides are inserted into a carrier assembly with fluid-flow covers and the carrier assembly is loaded into the temperature control apparatus. A freshly-prepared specimen treatment solution consisting of 1 mg/ml Pronase (Life Technologies, Rockville, MD) in 0.01 M Tris•Cl pH 7.8, 0.001 M EDTA, and 0.1 % Triton X-100 (v/v) is added to the specimen surface and incubated at 37° C. for 5 minutes. The mixture is rinsed from the matrix by three 500 ul washes of dH_20 over 15 minutes. The *in situ* sample preparation method unmasks DNA within the fixed specimen and permits the nucleic acid of the specimen to be used as a template for transcription. Specimen DNA is denatured by saturating the specimen with 500 ul dH_2O and using a temperature profile that heats the carrier to 94° C and maintains 94° C. for about 2 min.

Chamber covers replace the fluid-flow covers and are sealed against the specimen. A temperature profile brings the slides to and maintains a primer annealing temperature of, for example, 55° C. while 50-100 ul of the nucleotide/primer mix and 5 Units of Taq DNA Polymerase (Boehringer Mannheim, Indianapolis, IN) are added to each specimen and the chamber channels are closed. The nucleotide mixture for example may contain 140 uM each dATP, dGTP and dCTP, 100 uM dTTP and 40 uM Digoxigenin-11-dUTP (Boehringer Mannheim) in buffer (10 mM Tris•Cl, 50 mM KCl,

1.5 mM $MgCl_2$). In the one-step method at least one of the nucleotides is modified in order to detect incorporation. The primers are specific for a target sequence diagnostic for clinical specimens. A temperature profile for DNA amplification consists of, for example, ramping to and maintaining 55° C. for 30 seconds and ramping to and maintaining 94° C. for 10 seconds for 25 cycles, and ending with a profile of 10 min @ 72° C. Polymerase activity utilizes the target template within the nucleoid of cells each time the previous extension product melts under temperature denaturation and permits another primer molecule to bind and initiate polymerization.

After amplification, the chamber covers are removed and replaced with fluid-flow covers in order to rinse the specimens with dH_2O or detection buffer and start fluids moving through the carriers. The specimens are rinsed first by adding dH_20 and then 1 ml of TMN (40 MM Tris•Cl, pH 7.8, 6 mM $MgCl_2$, 5 mM NaCl) so that the fluids passed slowly through the diffusion layer between each specimen and its cover. Two ul of anti-Digoxigenin antibody conjugate from the GENIUS™ Detection System (Boehringer Mannheim) in 100 ul TMN are added to each specimen and incubated for 30 min at 37 °C. Two ml of suitable alkaline phosphatase substrate buffer is added slowly for 15 minutes to rinse unbound antibody conjugate away before adding alkaline phosphatase substrates, NBT (4.4 ul) and BCIP (3.3 ul) in 100 ul of the AP substrate buffer to the matrices. The reaction is incubated with a temperature profile of 1-2 hours at 35° C. and stopped by the addition of 2 ml dH_20 through the diffusion layer. Cellular structures in specimens may be counterstained. The carrier assembly is removed from the apparatus and the slides, or covers only, may removed from the carrier assembly for microscopic interpretation. The cells are observed under the microscope and both negative cells without the amplification label and positively-identified cells are visible. The amplified product is observed to remain within the target cells and enable such cells to be enumerated. The use of polymerase activity to amplify *in situ* increases detection sensitivity and enables the genetic entity to be tied to specific locations in the specimen.

If primers or nucleotides are not labeled during amplification, labeled oligonucleotides may be hybridized to amplification products for a 30-minute incubation, followed by rinsing twice at 5 minute intervals and once with slide at stringent temperature without zone spreading of signal from target cells. Standard aqueous hybridization buffers without formamide and standard SSC washes of 500 ul each may be used as known in the art. Detection systems utilizing biotin, digoxigenin,

fluorescence, antibodies or enzymes or combinations of these may be used. Labeled cells are visualized with standard light or fluorescent microscopy, depending upon which label is used.

In summary, the present invention provides an automated apparatus with means for heating and cooling and maintaining the specimen to or at any given temperature for a given time period, a computer means to generate signals that control said temperatures and times, and means to control evaporation of reagents added to the specimen for thermal cycling. While the invention has been described in detail with respect to specific illustrative examples and embodiments, it will be apparent that numerous other variations, modifications and embodiments are possible, and accordingly all such variations, modifications and embodiments as used by those of skill in the mechanical arts and related disciplines are to be regarded as being within the scope of the invention. Such variations include, but are not limited to the detection of proteins or other cellular components using known detection methods and reagents.

INDUSTRIAL APPLICABILITY

The invention provides evaporation-proof chambers and a temperature-controlled apparatus enabling *in situ* sample preparation, DNA amplification, DNA hybridization or other complexation of specific molecules within biological specimens for clinical diagnoses of said biological specimens, wherein said biological specimens are contained on microscope slides, specimen carriers or in a 96-well microplate.

BEST MODE OF THE INVENTION

An apparatus for providing temperature control to a specimen carrier comprises a carrier assembly and a temperature-controlled plate and control mechanisms. The carrier assembly consists of one or more specimen carriers, each of said specimen carriers comprising a carrier base and cover enclosing a thin compartment for holding a specimen and reaction fluids. The temperature-controlled plate has a first position in thermal contact with one side of said specimen carriers placed at said site and a second position out of said thermal contact with means for moving said temperature-controlled heating plate between said first position and second position. A fan provides for laminar air flow between said temperature-controlled plate and said specimen carriers when said temperature-controlled plate is in said second position. Temperature control means are provided to adjust the temperature of said temperature-controlled plate in response to control signals by computer means connected to said temperature control means and to said means for providing laminar air flow. When said temperature-

controlled plate is in the first position, the temperature of the specimen carriers may be brought to a first temperature through thermal contact with the temperature-controlled plate and when the temperature-controlled plate is in the second position, laminar air flow provided between the temperature-controlled plate and the specimen carriers may be used to bring the carriers to a second temperature.

CLAIMS

What is claimed is:

1. An apparatus for providing temperature control to a specimen carrier, comprising:

(a) one or more specimen carriers, each of said specimen carriers comprising a thin compartment for holding a specimen and reaction fluids;

(b) a temperature-controlled plate having a first position in thermal contact with one side of said specimen carriers placed at said site and a second position out of said thermal contact;

(c) means for moving said temperature-controlled heating plate between said first position and second position;

(d) means for providing laminar air flow between said temperature-controlled plate and said specimen carriers when said temperature-controlled plate is in said second position;

(e) temperature control means capable of adjusting the temperature of said temperature-controlled plate in response to control signals; and

(f) computer control means connected to said temperature control means and to said means for providing laminar air flow, wherein when said temperature-controlled plate is in the first position, the temperature of the specimen carriers may be brought to a first temperature through thermal contact with the temperature-controlled plate and when the temperature-controlled plate is in the second position, laminar air flow provided between the temperature-controlled plate and the specimen carriers may be used to bring the carriers to a second temperature.

2. An apparatus for providing temperature control to a specimen carrier according to claim 1, wherein the means for changing the location of the temperature-controlled plate comprises means for lifting the temperature-controlled plate into contact with the one or more specimen carriers and for lowering the temperature-controlled plate away from the one or more specimen carriers.

3. An apparatus for providing temperature control to a specimen carrier according to claim 1, wherein the first temperature is a temperature which denatures nucleic acid complexes and the second temperature is chosen to be just below the melting

temperature of primer oligonucleotides.

4. An apparatus for providing temperature control to a specimen carrier according to claim 1, further comprising sensing means in thermal contact with the temperature-controlled plate and connected to said computer means, wherein when a selected temperature is detected by said sensing means, said computer means causes a distance between the temperature-controlled plate and one or more specimen carriers to change; wherein when said selected temperature is detected, laminar air flow between said temperature-controlled plate and said one or more specimen carriers is provided by said means for providing laminar air flow; wherein said means for providing said laminar air flow comprises a fan directing air through a plenum into a laminar air flow space between said temperature-controlled plate and said one or more specimen carriers.

5. A method for providing temperature control to one or more specimens in one or more specimen carriers in a specimen treatment process, comprising:

(a) placing said one or more specimen carriers in an enclosure having having an interior space and a site for positioning one or more specimen carriers, each of the said one or more specimen carriers comprising a thin compartment for holding a specimen;

(b) providing:

(i) a temperature-controlled plate located in said enclosure, said temperature-controlled plate having a first position in thermal contact with one side of said carriers placed at said site and a second position removed from said thermal contact;

(ii) means for moving said temperature-controlled heating plate between said first position and second position;

(iii) means for providing laminar air flow between said temperature-controlled plate and said carriers when said temperature-controlled plate is in said second position;

(iv) temperature control means capable of adjusting the temperature of said temperature-controlled plate in response to control signals; and

(v) computer means connected to said temperature control means and to said means for providing laminar air flow, wherein when said

temperature-controlled plate is in the first position, the temperature of the one or more specimens may be warmed through thermal contact with the temperature-controlled plate and when the temperature-controlled plate is in the second position, laminar air flow provided between the one or more specimen carriers and the temperature-controlled plate may be used to cool the one or more specimen carriers;

(c) performing one or more temperature-changing steps in a predetermined sequence along with one or more specimen treatment steps, each of said temperature-changing steps being selected from the group consisting of:

(i) changing the temperature of said one or more specimen carriers from a warmer desired temperature by moving said temperature-controlled plate to said first position utilizing said means for changing the location of said temperature-controlled plate with respect to said site and providing control signals to adjust the temperature to said warmer desired temperature and to maintain the temperature of the heating plate at said warmer desired temperature for a first predetermined time period; and

(ii) moving said temperature-controlled plate to said second position utilizing said means for changing the location of the said temperature-controlled plate and one or more specimen carriers utilizing said means for providing laminar air flow to adjust the temperature of said temperature-controlled plate to a cooler desired temperature; and maintaining said cooler temperature for a second predetermined time period.

6. A device for detecting molecular targets in a biological specimen at temperatures elevated above ambient temperature, comprising:

(a) a specimen carrier base containing a biological specimen in a flat biological specimen area;

(b) a first cover covering a portion of said specimen carrier base and at least a portion of said flat biological specimen area;

(c) said specimen carrier base and said cover forming an enclosure, having an interior space in communication with said biological specimen,

comprising an evaporation-controlled chamber for reactions involving molecular detections with said biological specimen;

(d) at least one opening in said cover for adding reaction fluids to said evaporation-controlled chamber and removing reaction fluids from said chamber; and

(e) means for providing a closure to said opening in said cover that is both a water vapor barrier and pressure relief for said evaporation-controlled chamber.

7. A device for detecting molecular targets in a biological specimen according to Claim 6, further comprising a means to make a temporary seal between said cover and a discrete area of said biological specimen fixed on said specimen carrier base when said cover and said specimen carrier base are brought together to form said evaporation-controlled chamber; wherein the said means to make said seal is selected from the group consisting of spring tension, gaskets, conformal coatings and adhesives; and wherein the said temporary seal is disengaged when said cover and said specimen carrier base are separated.

8. A device for detecting molecular targets in a biological specimen according to Claim 6, further comprising the said biological specimen fixed on a specimen carrier base; wherein said specimen carrier base is optically clear in the said flat biological specimen area for microscopic evaluation of the said molecular targets in the said biological specimen.

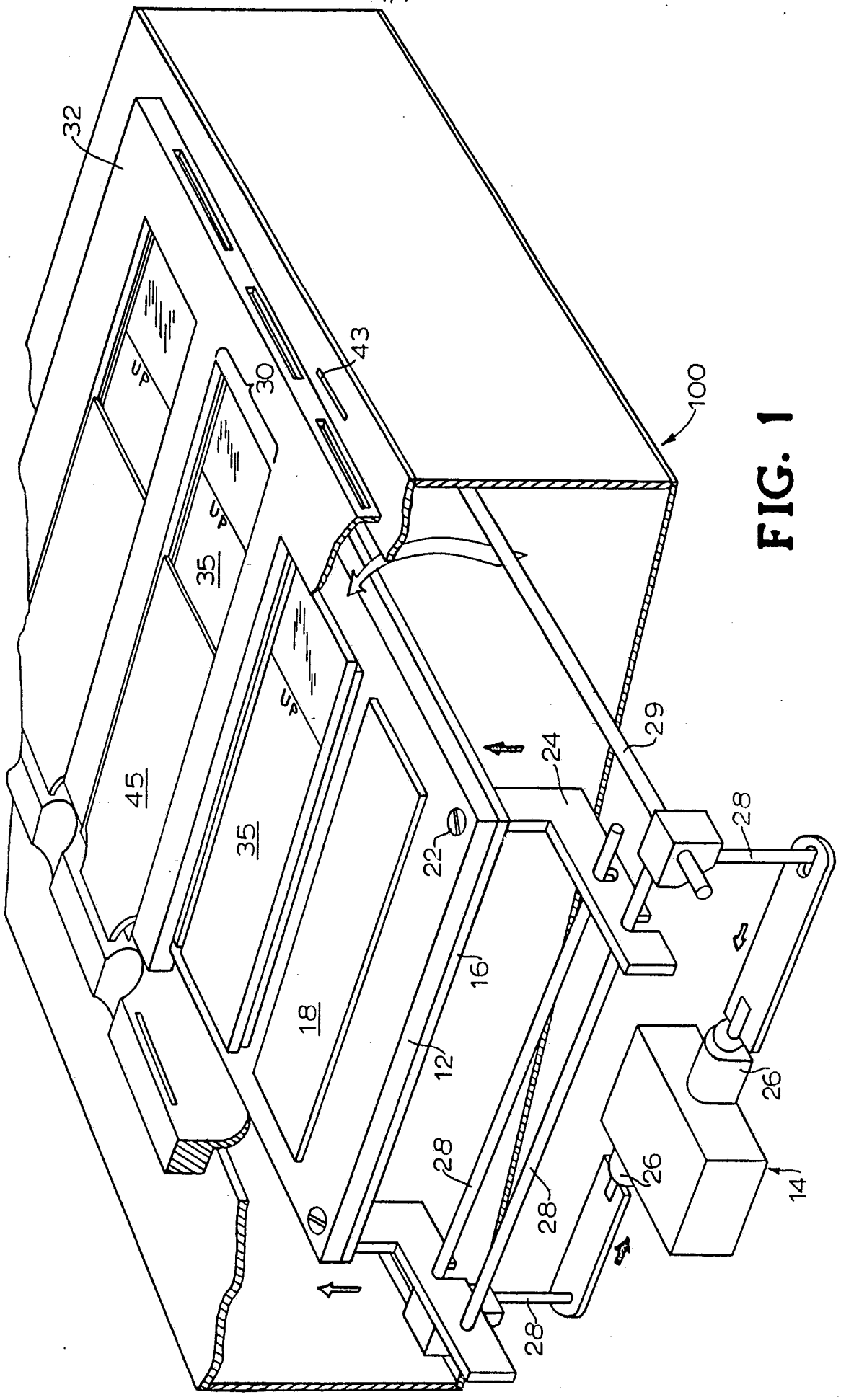

FIG. 1

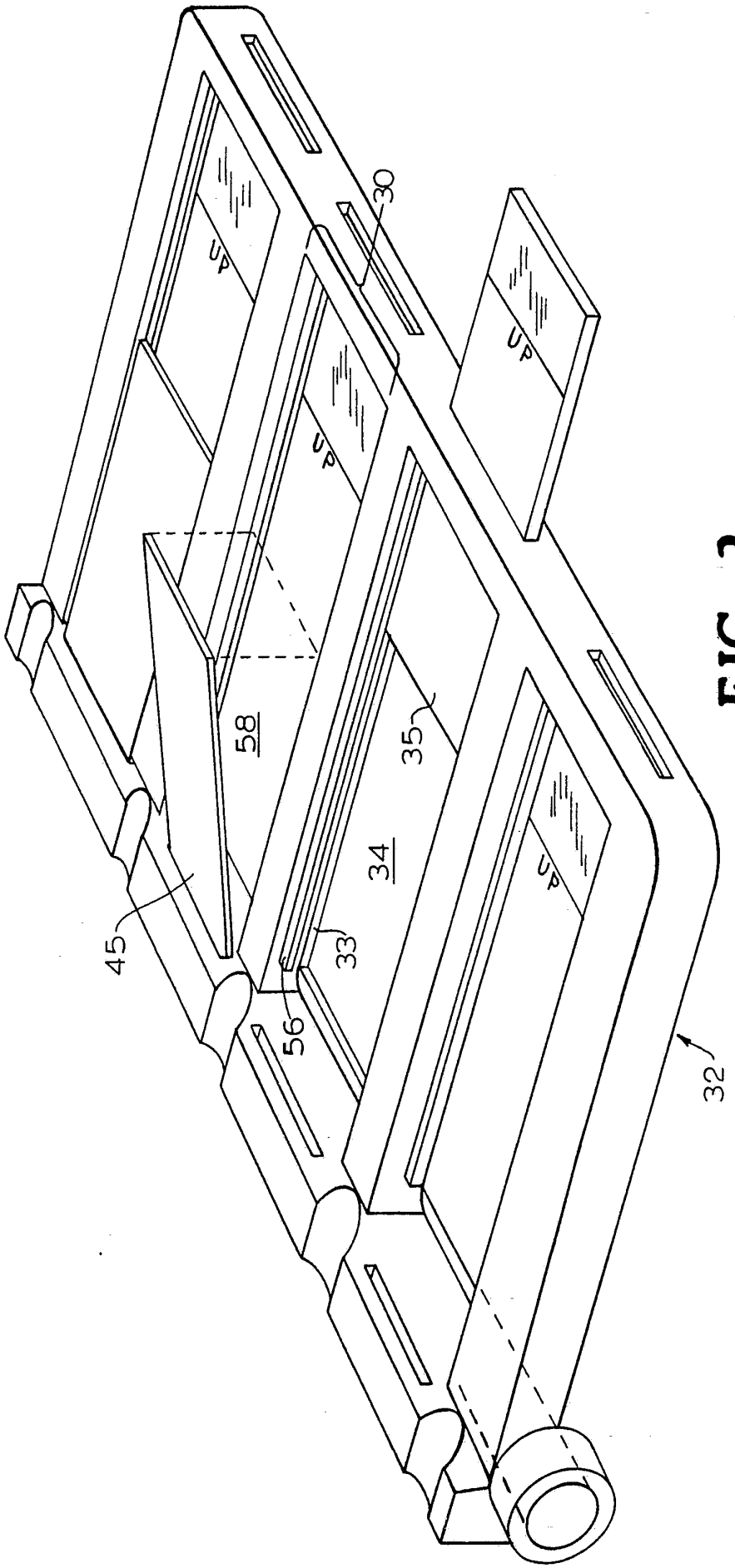

FIG. 2

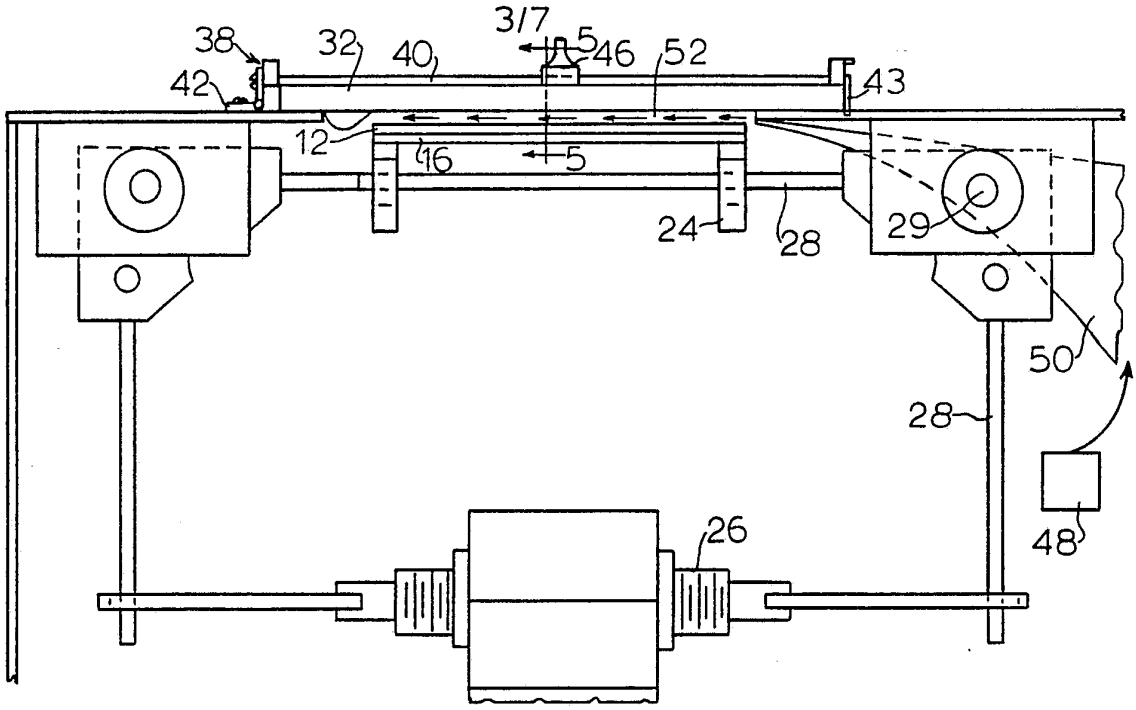

FIG. 3

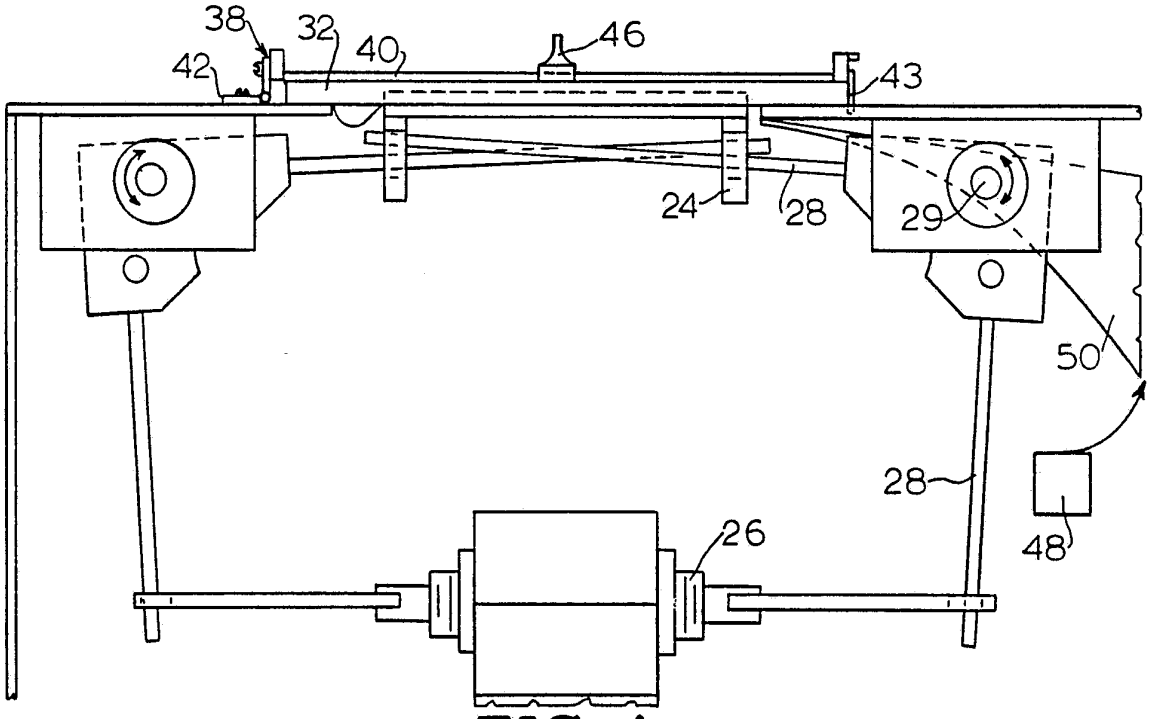

FIG. 4

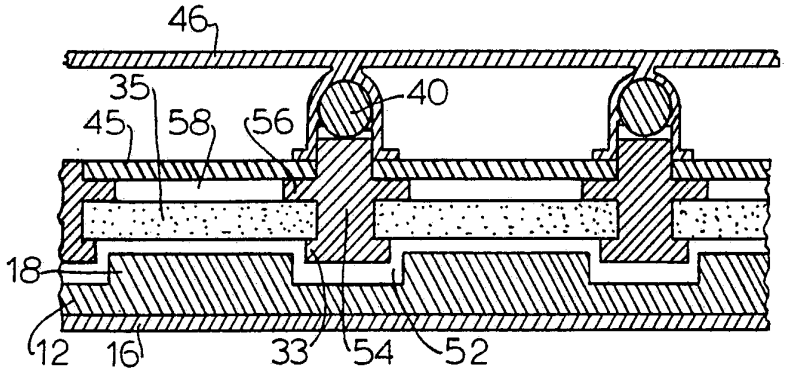

FIG. 5

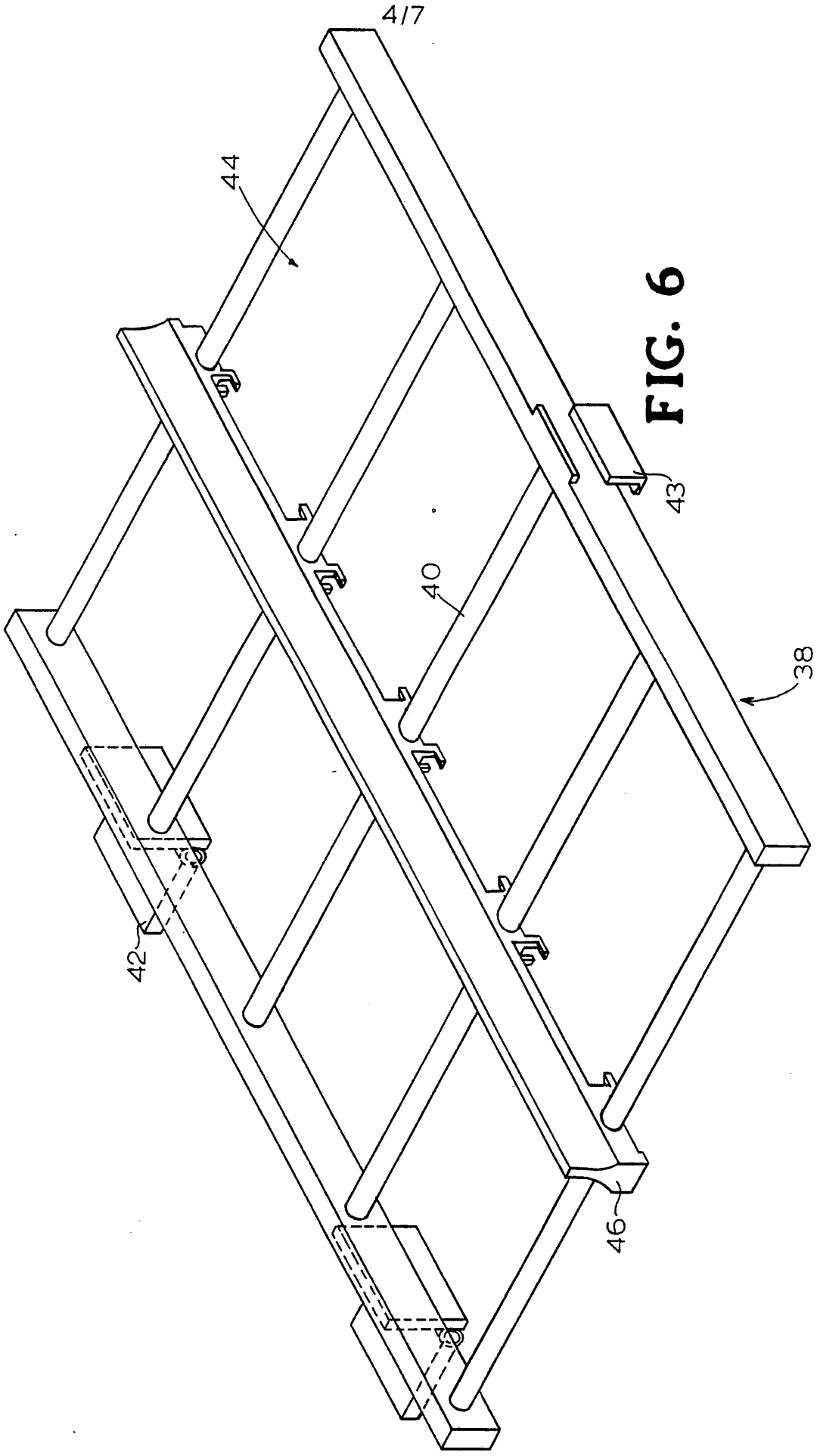

FIG. 6

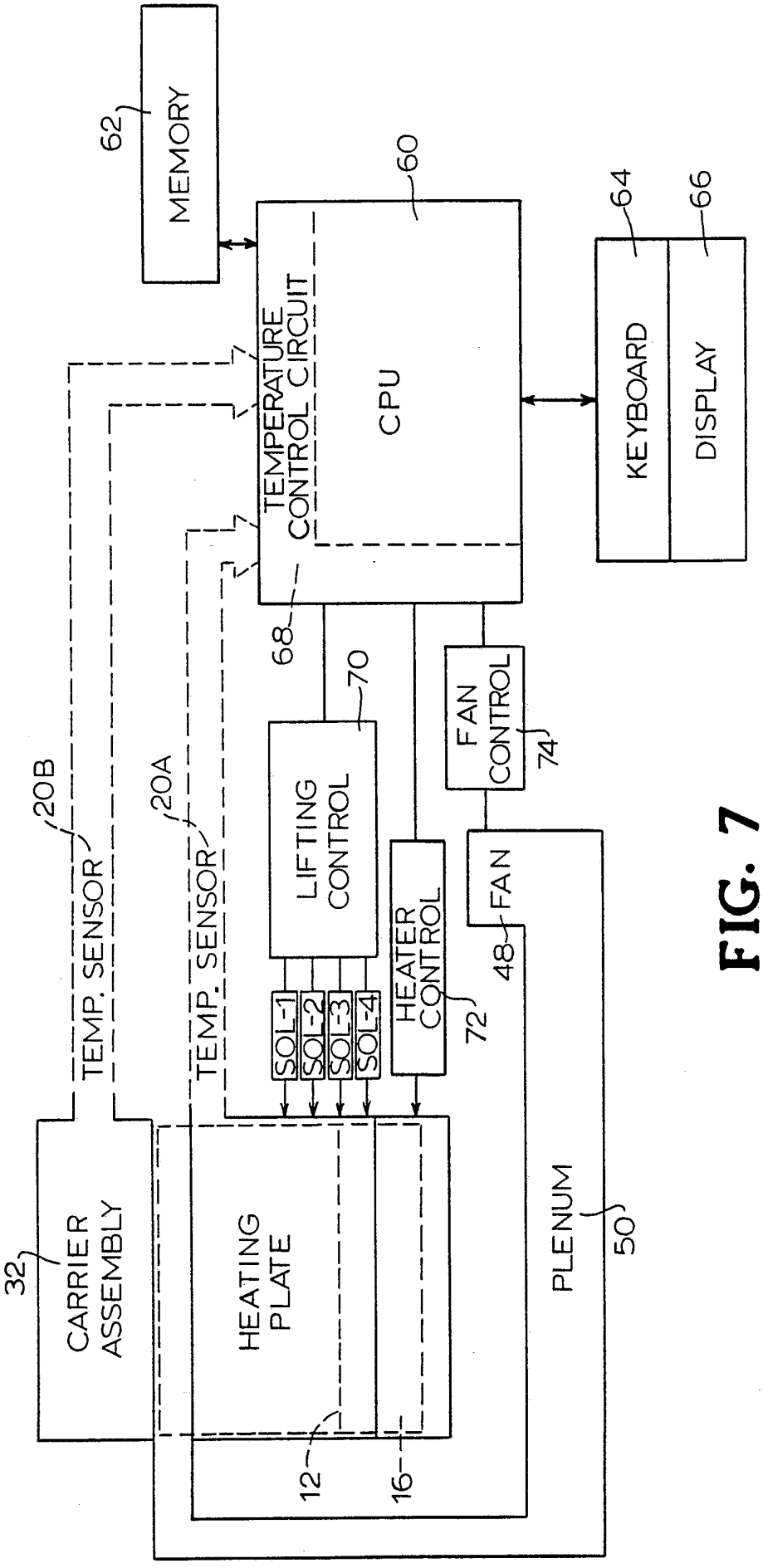
MEMORY
62
60
CPU
TEMPERATURE CONTROL CIRCUIT
64
KEYBOARD
66
DISPLAY
68
70
LIFTING CONTROL
FAN CONTROL
74
20B
TEMP. SENSOR
20A
TEMP. SENSOR
SOL-1
SOL-2
SOL-3
SOL-4
HEATER CONTROL
72
FAN
48
32
CARRIER ASSEMBLY
HEATING PLATE
PLENUM
50
12
16

FIG. 7

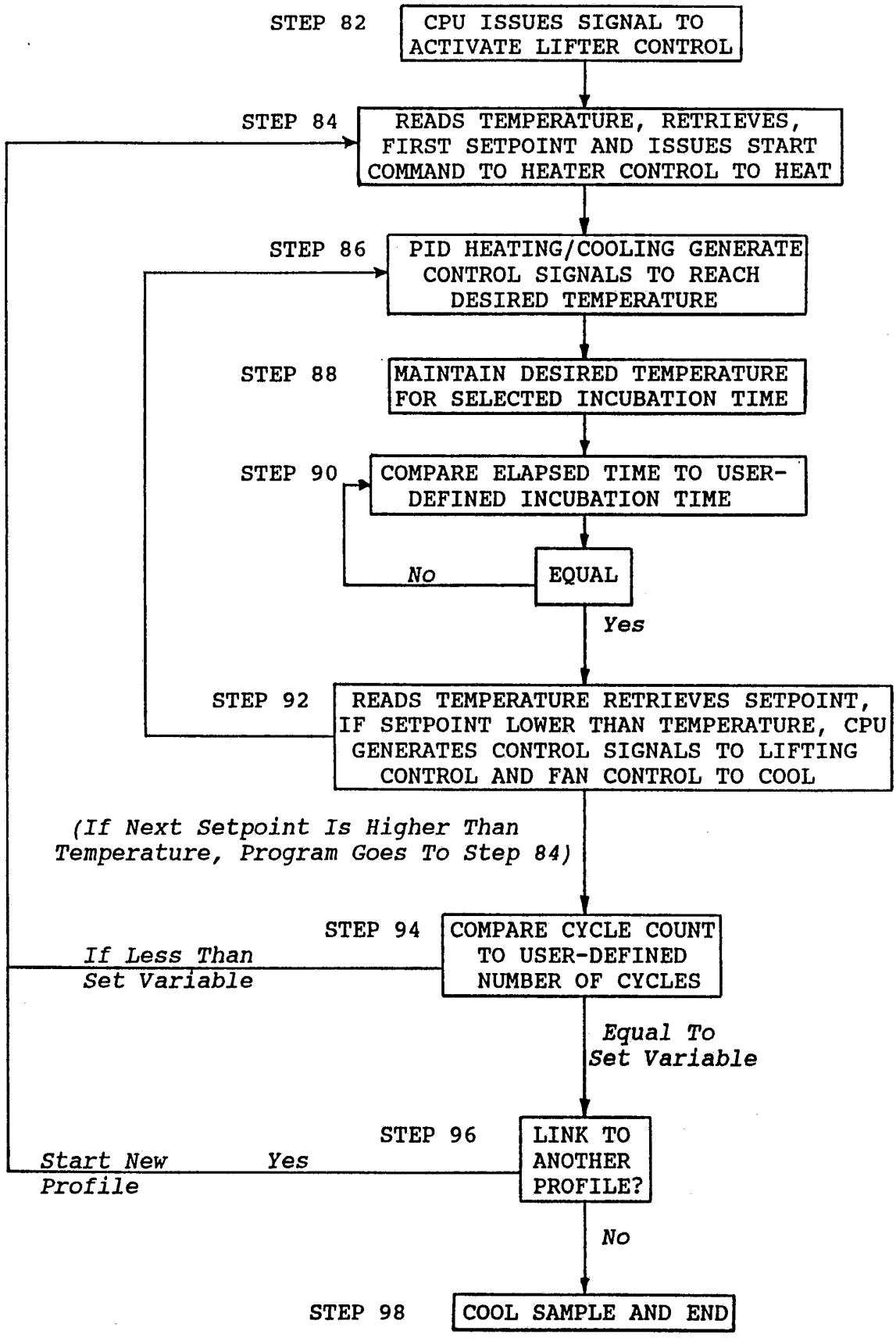
STEP 82
CPU ISSUES SIGNAL TO ACTIVATE LIFTER CONTROL
STEP 84
READS TEMPERATURE, RETRIEVES, FIRST SETPOINT AND ISSUES START COMMAND TO HEATER CONTROL TO HEAT
STEP 86
PID HEATING/COOLING GENERATE CONTROL SIGNALS TO REACH DESIRED TEMPERATURE
STEP 88
MAINTAIN DESIRED TEMPERATURE FOR SELECTED INCUBATION TIME
STEP 90
COMPARE ELAPSED TIME TO USER-DEFINED INCUBATION TIME
No
EQUAL
Yes
STEP 92
READS TEMPERATURE RETRIEVES SETPOINT, IF SETPOINT LOWER THAN TEMPERATURE, CPU GENERATES CONTROL SIGNALS TO LIFTING CONTROL AND FAN CONTROL TO COOL
(If Next Setpoint Is Higher Than Temperature, Program Goes To Step 84)
STEP 94
COMPARE CYCLE COUNT TO USER-DEFINED NUMBER OF CYCLES
If Less Than Set Variable
Equal To Set Variable
STEP 96
LINK TO ANOTHER PROFILE?
Start New Profile
Yes
No
STEP 98
COOL SAMPLE AND END

FIG. 8

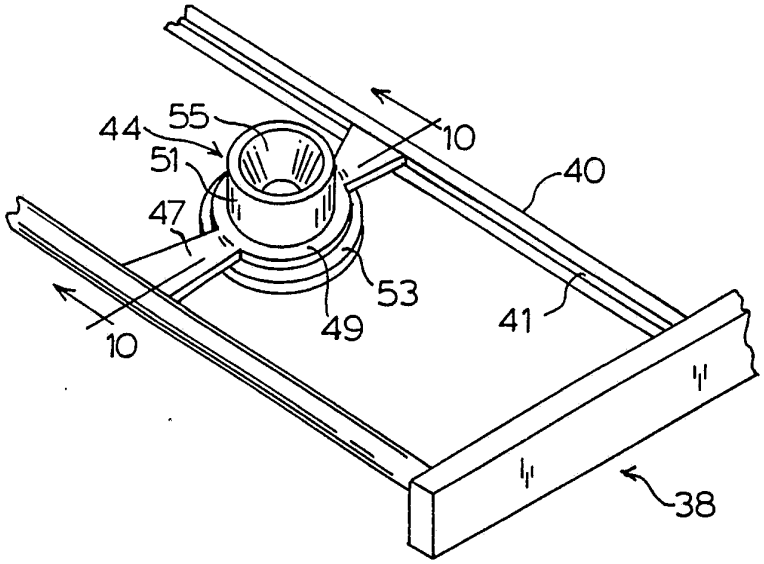

FIG. 9

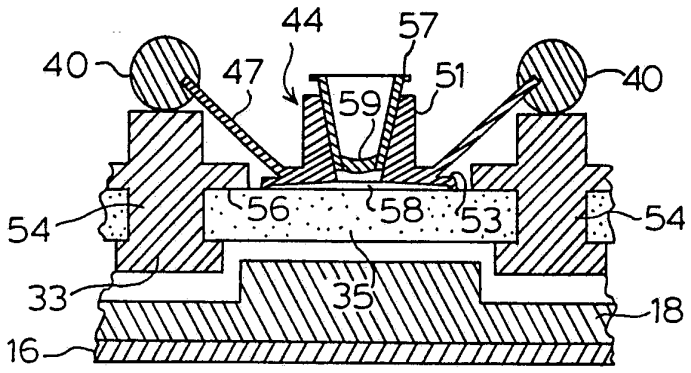

FIG. 10

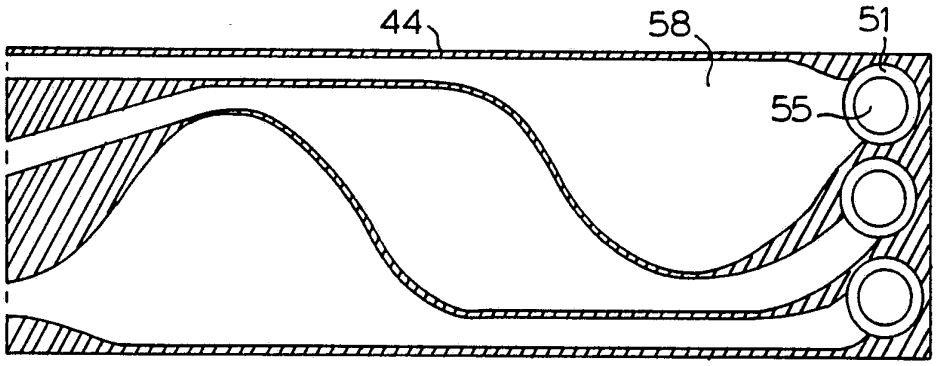

FIG. 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/02686

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12Q 3/00; C12M 1/02, 1/38; F28F 5/00
US CL :435/3, 287, 290, 316, 809; 165/61, 86
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/3, 284, 287, 290, 291, 298, 299, 316, 809; 165/61, 86; 219/200, 201, 443

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 3,556,731 (Martin) 19 January 1971, see entire document.	1-5
A	US, A, 4,384,193 (Kledzik et al.) 17 May 1983, see entire document.	1-5
A	US, A, 4,609,037 (Wheeler et al.) 02 September 1986, see entire document.	1-5
A	US, A, 4,865,986 (Coy et al.) 12 September 1989, see entire document.	1-5
A	US, A, 4,950,608 (Kishimoto) 21 August 1990, see entire document.	1-5

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
17 May 1993

Date of mailing of the international search report
09 JUN 1993

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. NOT APPLICABLE

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Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/02686

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 5,038,852 (Johnson et al.) 13 August 1991, see entire document.	1-5
A	US, A, 5,061,630 (Knopf et al.) 29 October 1991, see entire document.	1-5
A	US, A, 4,666,853 (Meserol et al.) 19 May 1987, see entire document.	6-8
A,P	US, A, 5,139,951 (Butz et al.) 18 August 1992, see entire document.	6-8
A,P	US, A, 5,149,501 (Babson et al.) 22 September 1992, see entire document.	6-8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/02686

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
(Telephone Practice)

I. Claims 1-5, drawn to an apparatus for providing temperature control, classified in Class 435/3.

II. Claims 6-8, drawn to an device for detecting molecular targets, classified in Class 435/287.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.