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(71) Applicant *(for all designated States except US)*: **MOLECULAR SENSING PLC** [GB/GB]; 3 Challeymead Business Park, Bedford Road, Melksham SN12 8LH (GB).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **CLARKSON, John, Michael** [GB/GB]; 3 Challeymead Business Park, Bradford Road, Melksham, SN12 8LH (GB).

(74) Agents: **BRADY, Paul, Andrew** et al.; Abel & Imray, 20 Red Lion Street, London WC1R 4PQ (GB).

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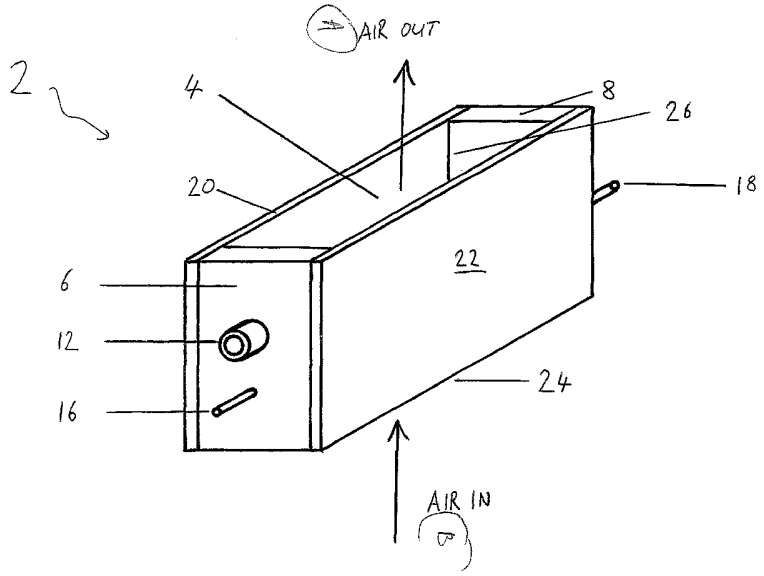
(54) **Title:** APPARATUS FOR HEATING AND/OR COOLING SAMPLES




(57) **Abstract:** The invention provides a fluid sample heating and/or cooling device comprising - a means for receiving a plurality of sample vessels, - a plurality of voids (4), each positioned to correspond with or enclose a corresponding sample vessels (12) when the sample vessel is in place, each void being provided with a heater means (16, 18) for heating air in the void, and - an air propulsion means arranged to move air through the voids, wherein the air in one void is thermally insulated from the air in one or more other voids.

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# APPARATUS FOR HEATING AND/OR COOLING SAMPLES

This invention relates to apparatus for diagnostic, experimental and other laboratory procedures and methods associated therewith.

A large number of diagnostic procedures include steps in which temperature changes are effected in a sample. Tight control over the temperature of a sample is required in order to achieve reproducible and accurate results. Accurate temperature control is beneficial in apparatus for various molecular biological applications, for example in apparatus for restriction digest experiments, isothermal or variable temperature amplification experiments, nuclease or protease digests, or protein expression experiments. Temperature is also important in many spectrometers. The tight control of the temperature of a sample may be required to achieve optimal enzyme activity, to ensure effective denaturation of double stranded nucleic acids or to enable correct annealing of oligonucleotides, for example oligonucleotide primers.

One molecular application in which controlled heating is particularly important is the polymerase chain reaction (PCR). The principle of the PCR nucleic acid amplification technique is described in US Patent US 4,683,195 (Cetus Corporation/Roche). Apparatus for carrying out the PCR reaction have been described in, for example, European Patent application EP 0 236 069 (Cetus Corporation/ Roche/PE). Such apparatus are commonly referred to as "thermocyclers".

Briefly, in a PCR reaction, a sample is subjected to a cycling between three phases:

1. Denaturation, during which a mixture of the target DNA, individual nucleotide bases (usually A,T,C and G), primers and a suitable DNA polymerase are heated to a relatively high temperature (typically over 80 °C) so that the two strands of the target DNA separate;
2. Annealing, during which the primers are allowed to anneal to the target DNA at a relatively low temperature (typically around 50 °C to 60 °C); and
3. Extension, during which the DNA polymerase synthesises strands of oilgonucleotides complimentary to the target strands at an intermediate temperature (typically around 70 °C).

In theory the quantity of target DNA present is doubled in each cycle. The cycle is repeated as many times as necessary to obtain a desired quantity of product, typically around 30 times.

The efficiency of a PCR amplification procedure is heavily dependent on the rates at which the sample is cycled between the various temperatures and the accuracy of the temperature control

and accordingly it is desirable for accurate, yet rapid, heating and cooling to be used. In certain thermal cycler devices of the prior art, there is a significant time lag before the majority of a sample reaches the target temperature. That time lag is commonly the rate determining step in the procedure.

With some reaction vessel geometries and reaction vessel materials, the variation of temperature across a sample can also be significant, leading to further inaccuracies and thus reaction inefficiencies. Temperature variations of up to 10 °C are common in the case of plastic 0.2ml or 0.5ml tubes suitable for biological reactions, for example tubes of the type commonly known as Eppendorf tubes.

It is efficient for diagnostic procedures to be carried out on many samples simultaneously in a parallel fashion. Accordingly, various devices have been developed for simultaneous thermal cycling of a plurality of samples. One example of such a device is the LightCycler® device, available from Roche Diagnostics (Roche Diagnostics Ltd., Bell Lane, Lewes, East Sussex, BN7 1LG, U.K.). A device with many of the features of the Lightcycler device is described in WO 97/46707 and WO 97/46712. In the Lightcycler device, a carousel having a plurality of sample tube receiving slots is located in an enclosed housing. The housing is in communication with a fan and a heater. In use, sample capillary tubes containing the samples of interest are inserted into the carousel. During a heating phase, the fan pushes hot air into the housing, causing the samples to be heated. During a cooling phase, the apparatus is vented and the fan pushes ambient air into the housing. An optical detection unit comprising a light source and a fluorescence detector is arranged to interrogate the contents of one capillary tube at a time along the length of the tube. The carousel rotates such that each sample tube may be aligned with the optical detection unit in turn.

The Lightcyler device enables the progress of several PCR reactions to be monitored simultaneously in "real time", whilst the reaction is still progressing. It also enables the kinetics of a reaction to be monitored.

In WO01/35079 there is described a combined fluorometer and thermal cycler in which several samples may be analysed for fluorescence characteristics simultaneously. The fluorometer comprises a plurality of low heat-generating light sources, means for positioning a plurality of containers for containing potentially fluorescing sample into optical communication with said

light sources, wherein each light source corresponds with one of said containers when in position, a first optical path means for guiding light from said light source to said corresponding container, an optical signal sensing means in optical communication with the sample in said positioned containers, and a second optical path means for guiding emitted light from the sample to said optical signal sensing means.

One low heat-generating light source is provided for each of the containers in one-to-one correspondence. The low heat-generating light sources are defined as light sources operated at a level below the level at which active cooling of the light source, such as via a fan, is required. The low heat-generating light sources provide adequate power to the samples because light is not wasted on the spaces between the positioned containers. The thermal cycler portion of the device of WO 01/35079 comprises a thermally controlled base having a plurality of wells for receiving sample containers, the base being fabricated on a thermoelectric heater/cooler element, and a thermally controlled cover having a plurality of apertures. The thermally controlled cover may be an electrically heated plate. A device according to WO 01/35079 is available from M J Research, Inc., 590 Lincoln Street, Waltham, MA 02451, U.S.A.

The major disadvantage of the LightCycler device or devices of the type described in WO01/35079 is that all of the samples are subjected to the same single temperature profile. It is not possible to apply different temperature profiles to different samples at the same time. Various devices have been designed to enable samples to be analysed simultaneously with different thermal profiles. One such device, the SmartCycler®, is available, from Cepheid (Cepheid, 904 Caribbean Drive, Sunnyvale, CA 94089, U.S.A.). The Smartcycler® device comprises several individual modules (so-called I-Core® modules) each module being arranged to receive a single sample. Each module includes its own heating/cooling fan and its own optical excitation and detection unit. In effect, each module is a separate single sample thermal cycler. In use, as many modules as required are positioned in a housing block and, within the block, each module is controlled independently.

In WO 98/24548 there is disclosed a reagent vessel comprising an electrically conducting polymer capable of emitting heat when an electric current is passed through it. In one embodiment, the vessel is a box comprising a plurality of receptor bays, each bay comprising a polymer heater sheath with electrical connections such as to permit different electrical power to be supplied to each of the receptor bays. Separate tubes containing the samples are provided for

introduction into the bays. Because each receptor bay has a relatively large thermal mass, a device of WO 98/24548 does not allow rapid heating and cooling of a sample.

Accordingly, there remains a need for a device in which a plurality of samples may be subjected simultaneously to temperature variation cycles which are not the same for each sample and in which temperature changes are effected rapidly and accurately.

The invention provides a fluid sample heating and/or cooling device comprising
- a means for receiving a plurality of sample vessels,
- a plurality of voids, each positioned to correspond with or enclose a corresponding sample vessel when the sample vessel is in place, each void being provided with a heater means for heating air in the void, and
- an air propulsion means arranged to move air through the voids,
wherein the air in one void is thermally insulated from the air in the one or more other voids.

The invention offers the possibility of a relatively simple and versatile device which is suitable for use in molecular diagnostics applications, clinical analysis or other analysis applications or chemical or biochemical synthesis applications. The device of the invention is particularly suitable for use in molecular biology applications, especially PCR applications. Other applications in which the device of the invention offers particular advantages are synthesis applications, restriction digestion procedures, sequencing procedures, ligation procedures and DNA or RNA sizing procedures.

In the device of the invention, an individual air stream is used to heat or cool each sample. Preferably, at least one void is provided by an air duct. More preferably, each void is provided by an air duct. An air duct has the function of guiding air from the air propulsion means to a sample and may take any appropriate shape without restrictions as to its length, width or cross-sectional form.

The air in a void is thermally insulated from the air in another void or the other voids. Air in a void is considered to be insulated from air in another void if the thermal insulation is sufficient for the temperature of the air in one void not to significantly affect the temperature of air in the other void during operation of the device in the normal temperature range. Typically, samples are subjected to temperatures of from room temperature to 100 degrees C. Good thermal insulation

enables accurate individual control of the temperature of each sample. Good thermal insulation allows a sample to be maintained at a low temperature whilst another adjacent sample is maintained at a high temperature. Preferably, when the device is in use, the temperature of the air in one void causes the temperature in an adjacent void to be altered by less than 3 degrees C, preferably by less than 2 degrees C, more preferably by less than 1 degree C, for example by less than 0.5 degrees C.

Typically the thermal insulation is provided by the presence of a body of air or other poorly thermally conducting substance (or a vacuum) between the materials defining two neighbouring ducts. In one embodiment, the voids are provided by ducts that are hollow and the space between the ducts contains insulating air. Insulating air may be enclosed and stationary. Alternatively, and preferably, the insulating air is dynamic. Dynamic insulating air is preferably moved by an air propulsion device. For example, the insulating air in the space between the ducts may be pushed or pulled by the same fan that moves the air through the voids in which the sample vessels are located during use of the device of the invention. Dynamic air effectively thermally insulates one void from a neighbouring void or voids. Heat transmitted to the outside of the duct defining one void is carried away in the dynamic insulating air stream rather than being transmitted to a neighbouring void.

In another embodiment, the material defining two neighbouring ducts is itself an effective insulator and no space between the ducts is necessary. The material may be an effective insulator by virtue of its shape or bulk or it may be an effective insulator by virtue of its material properties (or a combination of the two).

For the heating of a sample, the heater means associated with the corresponding duct is activated so that the air stream passing the heater means is heated. By the action of the air propulsion means, the heated air is propelled past the sample. Air has a relatively low heat capacity and it can accordingly be heated and cooled rapidly. This enables rapid heat transfer to the sample vessels.

The heater means may, for example, be an electrically resistive heater element, for example a metal heater element. The heater means may be a ceramic heater. The heater means may be located around the periphery of the particular air duct or it may be located within the air duct, or

both. Because of the greater surface area available for contact with the air in the duct, it is preferred for the heater to be within the air duct.

Preferably, the heater means is a metal coil or a metal mesh. The metal may be an alloy. Most preferably the heater means is a metal coil. The dimensions of the coil should be such that the coil has an electrical resistance such that a current passing through the coil generates a power output sufficient to heat the passing air rapidly. The dimensions of the coil should also be such that the coil does not become so hot that it melts or becomes difficult or impossible to control accurately. Preferably, the resistance of the electrically resistive heater element does not vary so much over the operational temperature range of the device as to affect the controllability of the heater. The operational temperature range for the sample vessel is typically from 50 to 95° in the case of a PCR application.

To obtain a heater coil with resistance in the required range, it may be appropriate to connect two or more supplied heater coils together in series or in parallel, typically in series.

In many applications, the temperatures to which the samples are to be cooled are ambient or above ambient and it is not necessary for the cooling means to cool the samples to below ambient temperature. Accordingly, ambient temperature air is sufficiently effective in cooling the samples. If more rapid cooling or cooling to below ambient temperature is required, an additional, active, cooling means may be present in the device. Such a cooling means is arranged to be activatable during a cooling phase such that cold air (i.e. colder than ambient) reaches the sample. Such a cooling means may be in the duct with the heater means or it may make use of a dedicated cool air duct. In the case of a cooling means in the same duct as the heater, the cooling means may be, for example, a cold coil cooled by circulation of cooling fluid within it, or a thermoelectric device (e.g. a Peltier device). In the case of a cooling means in a dedicated cool air duct, the cooling means may be, for example, a cold finger containing ice, dry ice or some other cold material.

Preferably, the air propulsion means is a fan. Suitable fans are known in the art. The fan may be arranged to push or pull air past the sample vessels. Preferably, the fan is arranged to push air past the sample vessels. To enable a constant and consistent air flow to the samples, the fan is advantageously arranged to propel air into a holding chamber. As the outflow from the holding chamber is impeded, the air pressure in the holding chamber increases above ambient atmospheric pressure. Optionally, an air pressure monitor may be present in the chamber to

provide the operator with a reading of the air pressure. The pressure monitor may be used to regulate the fan by a feed-back mechanism. Air passes from the holding chamber to the ducts.

In an alternative embodiment, a fan is arranged to pull air past the sample vessels. To enable a constant and consistent air flow past the samples, the fan may be arranged to withdraw air from a holding chamber, the holding chamber being in communication with the sample ducts. As the inflow of air into the holding chamber is impeded, the air pressure in the holding chamber decreases below ambient atmospheric pressure. Optionally, an air pressure monitor may be present in the chamber to provide the operator with a reading of the air pressure. The pressure monitor may be used to regulate the fan by a feed-back mechanism. Air passes into the holding chamber from the ducts.

Preferably, the device of the invention comprises a single fan for all of the air ducts. That offers simplicity in construction and maintenance. The fan may be arranged to be permanently operational when the device is in use. The air flow at each sample container is thus continuous and it is merely the temperature of the passing air that varies with time.

The device of the invention is preferably operated in an automated fashion. Accordingly, the device preferably further comprises a control means for controlling each heater means.

Suitably the control means comprises a computer. The control means is preferably arranged to receive instructions from an operator regarding the required temperature of a given sample. For many molecular biology applications, including, for example, PCR, the temperature of a sample is varied with time. In that case, the control means is arranged to receive instructions from the operator regarding the required temperature profile over time for a particular sample. Preferably, the control means is arranged to control the temperature of each sample independently.

Preferably, the device of the invention further comprises a temperature sensor for determining the temperature of a sample vessel or the temperature of a sample in a sample vessel. The temperature sensor may be present in the sample vessel and be in direct physical contact with the sample. In that arrangement, the close physical contact enables a very accurate assessment of the temperature of the sample to be made. Alternatively, the temperature sensor may be present in the air duct in the proximity of the sample vessel. In that arrangement, the temperature sensor is of a more simple construction and thus cheaper and simpler to manufacture and maintain. The

temperature experienced by a temperature sensor in an air duct may not be the same as the actual temperature of the sample. However, with information from calibration experiments, the temperature of a temperature sensor in an air duct provides a measurement from which the temperature of the sample may be inferred. The temperature sensor may, for example be a platinum resistive thermometer, a thermistor, a thermocouple, for example a small semi-conductor sensor.

Preferably, the device comprises an individual temperature sensor for each sample vessel. Preferably, the temperature sensor provides to the control means an input signal representing the temperature of a sample or sample vessel and the control means provides an output to the heater means dependent on the input signal and a preset required sample temperature. The temperature sensor may be arranged to act as a safety feature. For example, it may be arranged to ensure that the temperature of given sample does not exceed a pre-set temperature limit, above which the sample or some part of the device may be damaged.

Suitably, the device also comprises a means for sensing the temperature of the heater means. That means may be a separate thermal sensing means or, for example in the case of a resistive heater, the heater itself may act as the thermal sensing means. Typically, the heater may be operated in a pulsed fashion such that, in a first phase, the heater is supplied at a high power level and acts primarily as a heater. In a second phase, the heater is supplied at a low power level and heat output is relatively modest. Instead, the applied voltage and current flow are both measured and the heater acts primarily as a resistance meter. The resistance of the heater is a measure of the temperature of the device and with suitable calibration that measurement may be converted into a reading of the temperature of the heater means. The reading of the temperature of the heater means may be used to activate a safety cut out mechanism. Such a mechanism is typically arranged to switch off the heater if its temperature exceeds a preset safety limit. With suitable calibration, the temperature of the heater may also or alternatively be used to infer the temperature of the sample vessel and/or the sample in the sample vessel.

The heater means are typically powered by a connected power source. In the case of an electrically resistive heater, the power source is an electricity supply.

Preferably, the device of the invention further comprises an optical detection means for detecting the optical characteristics of a sample. The optical characteristics may, for example, be fluorescence or UV-visible absorption characteristics.

The light source may be of a type known in the art. Suitable light sources include Light Emitting Diodes (LEDs), lasers and conventional bulbs, including halogen bulbs. The light source may produce light of a single wavelength, of a number of single wavelengths or of a mixture of wavelengths.

Preferably, the light source is an LED. In view of the fact that a strong output from the LED is generally required, the LED is generally operated at a high power level and it may become hot. Locally, the LED may reach temperatures of over 100°C. A raised temperature of the LED interferes with its performance causing it to emit light inconsistently and to fail sooner than would be the case under operation at a lower temperature. To control those problems and to enhance the performance of the LED, it is preferred for the LED to be cooled. Preferably, the LED is cooled all the time the device is switched on.

The LED may be cooled, for example, by its being attached with good thermal contact onto a plate, for example a metal plate. In turn, the plate may be cooled by a fan, a Peltier device or a heat conducting strip, for example a copper strip, in contact with a cold body.

Fluorescence-based approaches to real-time measurement of PCR amplification products have been proposed and are in common usage. Some such approaches have employed double-stranded DNA binding dyes (for example major or minor groove binding intercalating dyes, for example SYBR Green I (RTM) or ethidium bromide) to indicate the amount of double stranded DNA present. Other approaches have employed probes containing fluorescer-quencher pairs (for example the "TaqMan" (RTM) approach) that are cleaved during amplification to release a fluorescent product the concentration of which is indicative of the amount of double stranded DNA present. Such fluorescer-quencher pairs methods typically make use of fluorescence resonance energy transfer, (FRET), for example in a dual probes system (for example the "HYB-Probes" system). Adaptations of those approaches are known (as described in, for example, WO 95/30139), in which two or more dyes are used.

The device or holder of the invention is particularly suitable for use with the above-mentioned fluorescence systems. Commonly used emission wavelengths include 530nm (fluorescein), 640nm (LC Red 640) and 710 nm (LC Red 705).

It is also common to detect the presence of a particular amplification product by means of hybridisation probes. Such probes may be provided with fluorescent dyes with a variety of emission characteristics and, in a given experiment, it may be desirable to use more than one dye. The device of the invention is also suitable for use in such detection systems. The ability to analyse a plurality of wavelengths of light without the need for moving parts is particularly advantageous for such applications.

The device of the invention may be arranged to receive any convenient number of sample vessels. Preferably, the device is arranged to receive from 2 to 384 sample vessels, more preferably from 3 to 96 sample vessels, still more preferably from 5 to 20 sample vessels, for example 12 sample vessels.

In one embodiment of the device of the invention, the device is arranged to receive a sample holder unit, the sample holder unit comprising a plurality of sample vessels or sample vessel receiving spaces each for receiving a sample vessel.

The sample holder unit may be supplied with sample vessels in place, or it may be supplied with sample vessel receiving spaces. The sample holder unit may be provided with a stand such that the unit can be positioned steadily for sample loading without it being necessary for the operator to touch the sample vessels. The stand is preferably made of a transparent material or comprises voids such that samples vessels in the holder are visible to the operator whilst a sample is being deposited in a sample vessel. Contact of the operator with the sample vessels risks breakage of the vessel, contamination of the sample or the leaving of fingerprints on the sample vessel, which may interfere with optical assessment of the sample.

For user convenience an apparatus preferably comprises more than one device of the invention, each device of the invention optionally being independently operable within the apparatus. For example, an apparatus of the invention may comprise two devices of the invention. An apparatus may thus comprise two independent devices with two fans, two optical detection units, and two

arrays of sample receiving spaces. Alternatively, two devices in an apparatus may share a single fan. It is also possible for two devices to share a single detector.

The sample vessels are preferably tubular with a diameter in the range of from 0.5 to 10mm and with a sealed end and an open end arranged to receive a closure means. They are preferably transparent. Suitably, the sample vessels are made of glass or a plastics material. Optionally, the sample vessels comprise a portion of greater width at the closed end for receiving the closure means. The wider portion is preferably sufficiently long to receive and hold in place the closure means. The diameter and length of the closure means is preferably matched to the diameter of the wider portion of the sample vessel such that an air tight closure is obtained when the closure means is inserted into the sample vessel and the closure means acts as a stopper. Insertion of the stopper accordingly causes any air in the sample vessel to be compressed, causing the air pressure in the sample vessel to be increased.

With such a pressurising stopper in place, sample fluid in a sample vessel is held at above atmospheric pressure. That causes the boiling point of the fluid to be increased and reduces the risk of degassing (release of air bubbles) during heating, which may cause spillage.

Degassing of a sample solution (also known as "bumping") may, if not controlled, lead to sudden, violent eruptions of bubbles from the body of the sample solution which may result in spillage if the sample tube is not securely sealed. Even if the sample tube is securely sealed, degassing may result in redistribution of sample fluid to non-ideal parts of the sample tube. For example, a part of the sample may be moved to a portion of the sample tube at which heating and/or cooling is not as efficient as at the portion of the tube in which the sample is intended to be located. Bubbles may also hinder spectrophotometric measurements of the sample. The risk of bubbles causing those problems is much reduced by the application of air pressure greater than atmospheric pressure. Suitably, pressure of from 1.5 to 3.0 atmospheres is built up by application of the closure means.

In a further aspect, the invention provides a method for simultaneously and independently heating a plurality of sample tubes, which method comprises

- placing each of the plurality of sample tubes in its own void, each void being furnished with a heater means, and
- propelling air in each void past the respective heater means to the respective sample tube.

Because each void is furnished with its own heater, the method of the invention enables each of the plurality of sample tubes to be heated in its own individual way.

The invention also provides a method for carrying out an analytical measurement of nucleic acid reactions that combine thermal control and integrated online detection using a device according to the invention.

Certain embodiments of the invention will now be described in more detail with reference to the accompanying figures in which:

Figure 1A shows a device comprising a heater coil, a duct and a sample tube in cross sectional view;

Figure 1B shows a device comprising a heater coil, a duct and a sample tube in perspective view;

Figure 2 shows an array of ducts as given in Figure 1;

Figure 3 shows a sample holder and sample holder stand suitable for use in a device of the invention;

Figure 4 shows a sample holder with sample tubes in place together with a closure means unit.;

Figure 5 shows thermal cycling temperature data obtained using a device of the invention;

Figure 6 shows thermal cycling fluorescent data obtained using a device of the invention.

Referring to Figures 1A and 1B of the drawings there is shown a single sample tube heating and/or cooling device referred to generally by reference numeral 2. In Figure 1A the device is shown in cross sectional view, in Figure 1B, the device is shown in perspective view. The device comprises a void 4 formed by two substantially parallel duct walls 6 and 8. Duct wall 6 comprises an opening 10 for receiving a sample tube 12. A heater coil 14 connected to a power supply (not shown) spans void 4 and its connections 16 and 18 for connecting to a power supply pass through duct walls 6 and 8 respectively. As seen in the perspective view in Figure 1B, void 4 is formed by the two duct walls 6 and 8 and two side walls 20 and 22. Side walls 20 and 22 are substantially parallel to each other and perpendicular to the duct walls 6 and 8.

In use, air propelled by a fan (not shown) enters void 4 at its lower aperture 24 and exits the void at its upper aperture 26. The moving air contacts heater coil 14, which is hot by virtue of a heating current being passed through it. The moving air becomes hot, and then passes to sample

tube 12 causing the sample tube to be heated. After moving past sample tube 12, the air exits the void at upper aperture 26.

If thermal cycling of sample tube 12 is required, the current passing through heater coil 14 is periodically turned off. When heater coil 14 does not have a current passing through it, the incoming air entering through aperture 24 remains at room temperature. When that room temperature air contacts sample tube 12, it takes heat away from tube 12 and the tube cools down.

In Figure 2 there is shown a device, indicated generally by the reference numeral 28 comprising an array of voids. Device 28 comprises a series of voids, five voids being shown, 4a, 4b, 4c, 4d and 4e. Each void is formed by two duct walls 6a and 8a, 6b and 8b, 6c and 8c, 6d and 8d and 6e and 8e respectively, and two side walls 20a and 22a, 20b and 22b, 20c and 22c, 20d and 22d and 20e and 22e respectively. Each side wall 20 is separated from the side wall 22 of the neighbouring void by insulating space 27. Side walls 22a and 20b and separated by insulating space 27a, side walls 22b and 20c and separated by insulating space 27b, Side walls 22c and 20d and separated by insulating space 27c and side walls 22d and 20e and separated by insulating space 27d. Air, pushed by the fan (not shown) moves through insulating spaces 27a, 27b, 27c and 27d.

In use, air propelled by a fan (not shown) enters each of voids 4a to 4e at its lower aperture 24a to 24e and exits the void at its upper aperture 26a to 26e as described above in relation to single void 4 shown in Figures 1A and 1B. Each heater coil 14a to 14e may be controlled independently so that each sample tube 12a to 12e may, if required, be subjected to a different heating regime.

Referring to Figure 3, there is shown a sample tube holder referred to generally as 30 and a sample holder stand referred to generally as 32. Sample tube holder 30 comprises a roughly rectangular plate 34 furnished with 12 sample vessel receiving holes 36a to 36l defined by circular apertures. Sample vessel receiving holes 36a to 36l are arranged in two lines of 6 holes each. Plate 34 is surrounded by an external wall 38 which protrudes above the level of plate 34. On one side of sample tube holder 30 there is located a protrusion 40 which serves to desymmetrise the sample tube holder. At each of the two ends of plate 34, and perpendicular to the plate there is attached an arm 42 and 44.

Sample holder stand 32 comprises a roughly rectangular base 46 and front and rear walls 48 and 50 which project perpendicularly from the base. At the ends of the two walls, the gap between the walls is shaped to receive arms 42 and 44 of sample tube holder 30. Protruding into the arm receiving space at each end of the sample holder stand is a clip 52 and 54 respectively for engaging with a notch in the respective arm.

In use sample tube holder 30 is initially engaged with sample tube holder stand 32. A sample tube 56 (not shown in Figure 3) is located in each of as many of the sample receiving holes as required. Sample tube 56 is protected from breakage or contact with the operator by the walls 48 and 52. After sample has been loaded into as many of the sample tubes as required, a sample tube sealing closure means 58 (shown in Figure 4) is pressed into the sample tubes to seal them, sample tube holder 30 is disengaged from sample tube holder stand 32 and moved to the designated slot in a device of the invention (not shown).

In Figure 4 there is shown a sample tube holder 30 with two sample tubes 56a and 56c in place together with multiple sample tube sealing closure means 58. Multiple sample tube sealing closure means 58 comprises an essentially planar backing member 59 and a protruding stopper member 60 for each sample tube. The lid 58 is made of a resilient material such that circular lip 62 on each stopper member 60 forms a tight seal when inserted into its respective sample tube. In use, insertion of a stopper member 60 into a sample tube causes the air in the tube to be compressed.

The following examples illustrates the invention further:

**Example 1: Use of a device of the invention in a PCR protocol**

Two different PCR reactions with different thermal cycling protocols were carried out in adjacent reaction tubes. The genes to be amplified and the primers used are as shown in Table 1.

**Table 1**

| Reaction | Gene | Forward oligo name | Reverse oligo name | PCR amplicon size (bp) |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| 1 | Human β-globin | GLOB267F | GLOB267R | 267 |
| 2 | Ade 1 (*S. cerevisiae*) | SADE650F | SADE750R | 650 |

The PCR assay contents for a 10 reaction master mix of the β-globin 267 bp reaction were as shown in Table 2:

**Table 2**

| Reagent | Stock concentration | Volume taken (μl) Single reaction | Volume taken (μl) 10 reactions |
|---|---|---|---|
| Hot Start Master Mix | 10x | 2.0 | 20.0 |
| $MgCl_2$ | 25 mM | 1.6 | 16.0 |
| Forward primer | 11.75 μM | 1.36 | 13.6 |
| Reverse primer | 9.35 μM | 1.71 | 17.1 |
| DNA | 10 ng $\mu l^{-1}$ | 1.0 | 10.0 |
| Water | | 12.33 | 123.3 |

The PCR assay contents for a 10 reaction master mix for the Ade 1 650 bp reaction were as shown in Table 3:

**Table 3**

| Reagent | Stock concentration | Volume taken (μl) Single reaction | Volume taken (μl) 10 reactions |
|---|---|---|---|
| Hot Start Master Mix | 10x | 2.0 | 20.0 |
| $MgCl_2$ | 25 mM | 1.6 | 16.0 |
| SADE650F (forward primer) | 10.22 μM | 1.96 | 19.6 |
| SADE**750R** (reverse primer) | 10.6 μM | 1.89 | 18.9 |
| DNA | 10 ng $\mu l^{-1}$ | 1.0 | 10.0 |

| | | | |
|---|---|---|---|
| Water | | 11.55 | 115.5 |

The PCR parameters for the β-globin 267 bp amplification were shown in Table 4:

**Table 4**

| Program | Temperature (°C) | Duration | Number of cycles |
|---|---|---|---|
| Initial Denaturation | 95 | 3 min | 1 |
| PCR | 95 | 1s | 40 |
| | 60 | 5s | |
| | 72 | 5s | |
| Melt denaturation | 95 | 1s | 1 |
| | 65 | 15s | |
| | 95 | 0s | |
| Cool | 40 | 30s | 1 |

The PCR parameters for the Ade 1 650 bp amplification were as shown in Table 5:

**Table 5**

| Program | Temperature (°C) | Duration | Number of cycles |
|---|---|---|---|
| Initial Denaturation | 95 | 3 min | 1 |
| PCR | 95<br>55<br>72 | 2s<br>5s<br>20s | 40 |
| Melt denaturation | 95<br>65<br>95 | 1s<br>15s<br>0s | 1 |
| Cool | 40 | 30s | 1 |

Thermal cycling data for three cycles of the protocol of table 5 are shown in Figure 5. Fluorescence data for a larger number of cycles of the same protocol are shown in Figure 6.

Satisfactory simultaneous amplification of Human β-globin and Ade 1 (*S. cerevisiae*) was achieved in a device of the invention despite the PCR conditions for the two amplifications being different.

**Example 2: Demonstration of thermal accuracy**

Using a device of the invention as illustrated in Fig.2 and the sample tubes having the configuration illustrated in Fig. 3, each sample receiving position (numbered A1 to A12 in a first device and B1 to B12 in a second device) was programmed to maintain a temperature of 60 degrees C for 5 minutes and then 95 degrees C for 5 minutes. Table 5 shows the actual temperatures recorded in the air void between the sample tube and the heater at the end each 5 minute period. The closeness of the recorded temperatures to the set respective set temperature, demonstrates the accuracy of the device.

**Table 5**

| Sample position | Recorded temperatures when target temperature set to 60°C | Recorded temperatures when target temperature set to 95°C | Sample position | Recorded temperatures when target temperature set to 60°C | Recorded temperatures when target temperature set to 95°C |
|---|---|---|---|---|---|
| A1 | 60.01 | 95.01 | B1 | 60.07 | 95.09 |
| A2 | 60.02 | 95.17 | B2 | 60.10 | 95.13 |
| A3 | 59.95 | 95.14 | B3 | 60.06 | 95.08 |
| A4 | 59.99 | 94.97 | B4 | 60.06 | 95.04 |
| A5 | 59.94 | 94.98 | B5 | 60.00 | 95.13 |
| A6 | 60.03 | 95.04 | B6 | 60.05 | 94.98 |
| A7 | 59.98 | 95.03 | B7 | 60.02 | 95.02 |
| A8 | 60,05 | 95.03 | B8 | 59.98 | 95.11 |
| A9 | 59,93 | 94.83 | B9 | 59.98 | 95.04 |
| A10 | 59.97 | 95.01 | B10 | 60.00 | 95.02 |
| A11 | 59.80 | 94.92 | B11 | 59.97 | 95.05 |
| A12 | 59.94 | 94.93 | B12 | 59.97 | 95.07 |

**Example 3: Thermal cross talk**

Using a device of the invention as illustrated in Fig.2 and the sample tubes having the configuration illustrated in Fig. 3, sample positions A2, A5, A8 and A11 were set to 60 degrees C. Sample positions A1, A3, A4, A6, A7, A9, A10 and A12 were set to 95 degrees C. In that configuration, each 60 degree sample tube was located between two 95 degree sample tubes. Table 6 shows the temperature recorded in at sample positions A2, A5, A8 and A11. It can be seen that despite being adjacent to two positions set to a substantially higher temperature, the effect of this on the temperature of positions A2, A5, A8 and A11 was only slight and the temperature was within 0.5 degrees of the set temperature..

**Table 6**

| Sample position | Set temperature | Recorded temperature |
|---|---|---|
| A1 | 95°C | |
| A2 | 60°C | 60.39°C |
| A3 | 95°C | |
| A4 | 95°C | |
| A5 | 60°C | 60.47°C |
| A6 | 95°C | |
| A7 | 95°C | |
| A8 | 60°C | 60.45°C |
| A9 | 95°C | |
| A10 | 95°C | |
| A11 | 60°C | 60.45°C |
| A12 | 95°C | |

CLAIMS

1. A fluid sample heating and/or cooling device comprising
- a means for receiving a plurality of sample vessels,
- a plurality of voids, each positioned to correspond with or enclose a corresponding sample vessel when the sample vessel is in place, each void being provided with a heater means for heating air in the void, and
- an air propulsion means arranged to move air through the voids,
wherein the air in one void is thermally insulated from the air in one or more other voids.

2. A device as claimed in claim 1 wherein at least one void is provided by an air duct.

3. A device as claimed in claim 2 wherein an air duct is separated from one or more other ducts by a body of air.

4. A device as claimed in any one of claims 1 to 3 wherein the heater means comprises an electrically resistive heater element.

5. A device as claimed in any one of claims 1 to 4 wherein the air propulsion means is a fan.

6. A device as claimed in claim 5 wherein the device further comprises a holding chamber arranged to receive air propelled by the fan and distribute air to the voids.

7. A device as claimed in any one of claims 1 to 6 further comprising a temperature sensor for determining the temperature of a sample vessel or the temperature of a sample in a sample vessel.

8. A device as claimed in claim 7 comprising a temperature sensor for each sample vessel.

9. A device as claimed in any one of claims 1 to 8 further comprising a control means for controlling at least one of the heater means.

10. A device as claimed in claim 9 wherein a temperature sensor provides to the control means an input signal representing the temperature of a sample or sample vessel and the control means

provides an output to the heater means dependent on the input signal and a preset required sample temperature.

11. A device as claimed in any one of claims 1 to 10 further comprising an optical detection means for detecting the optical characteristics of a sample.

12. A device as claimed in any one or more of claims 1 to 11 in which the plurality of sample vessels are arranged in an array.

13. A device as claimed in any one or more of claims 1 to 12 in which the means for receiving the plurality of sample vessels is a means for receiving a sample holder unit, the sample holder unit comprising a plurality of sample vessels or sample vessel receiving spaces each for receiving a sample vessel.

14. A method for simultaneously and independently heating a plurality of sample tubes, which method comprises
- placing each of the plurality of sample tubes in its own void, each void being furnished with a heater means, and
- propelling air in each void past the respective heater means to the respective sample tube.

15. A method for carrying out an analytical measurement of nucleic acid reactions that combine thermal control and integrated online detection using a device as claimed in any one of claims 1 to 13.

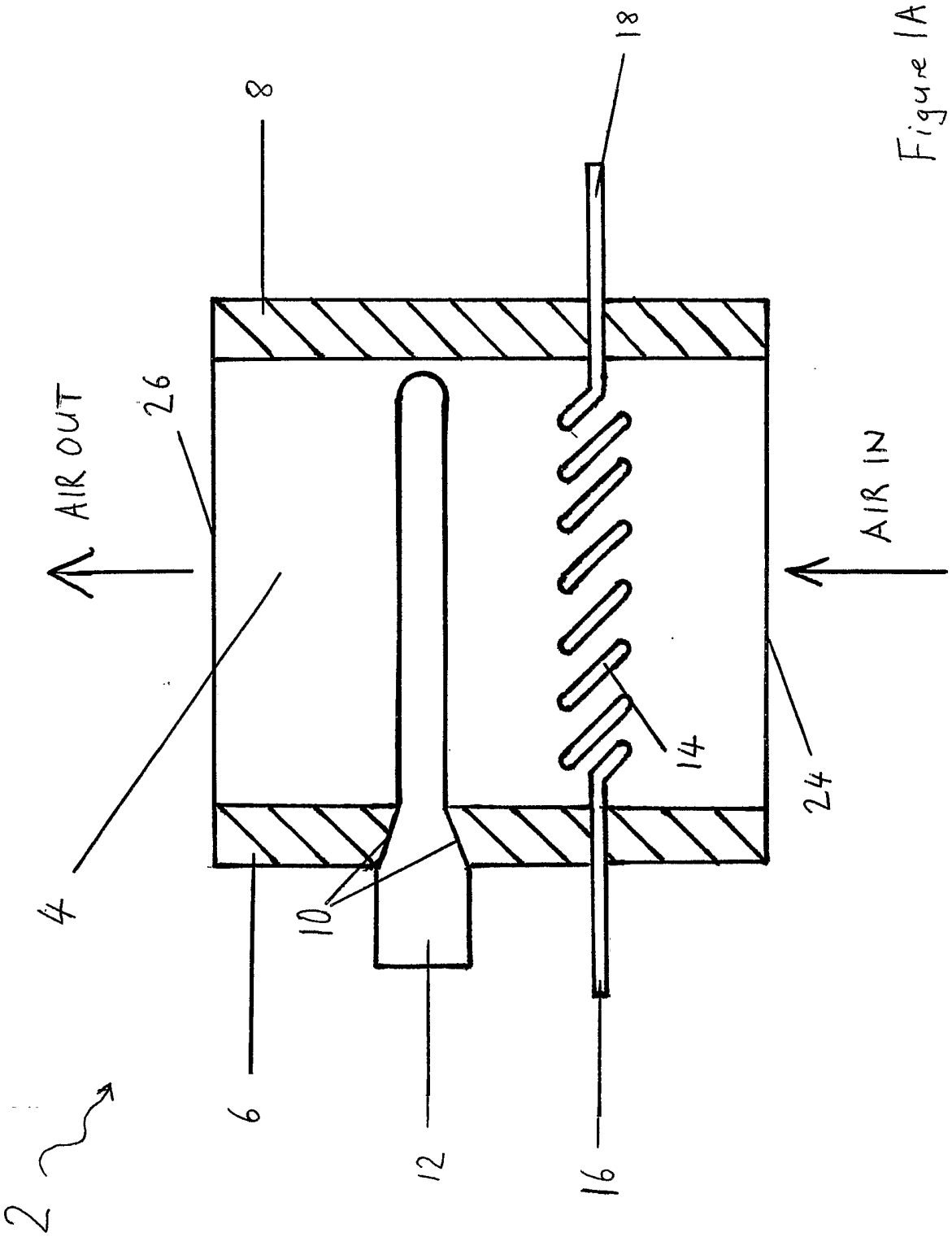



Figure 1A

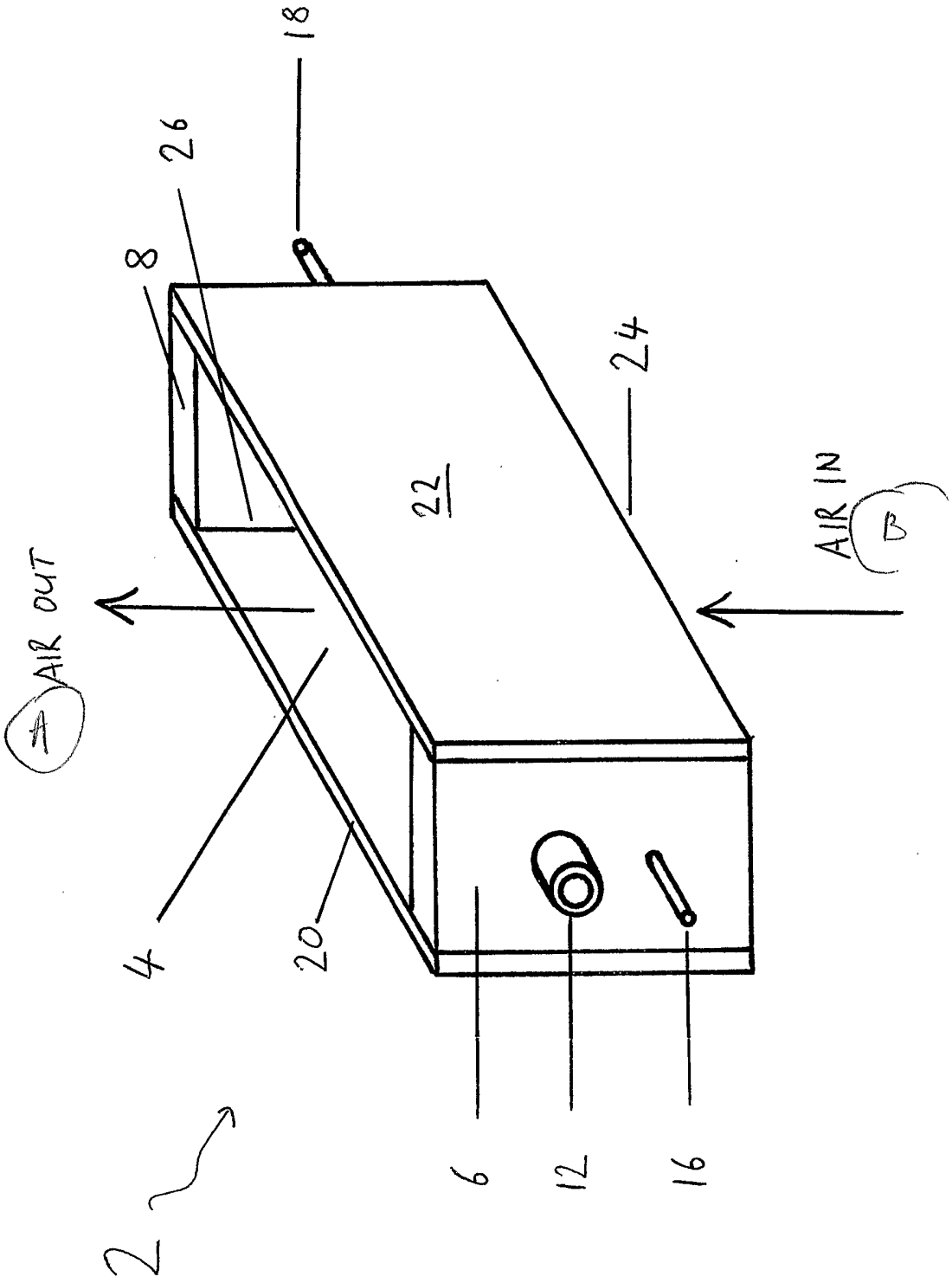
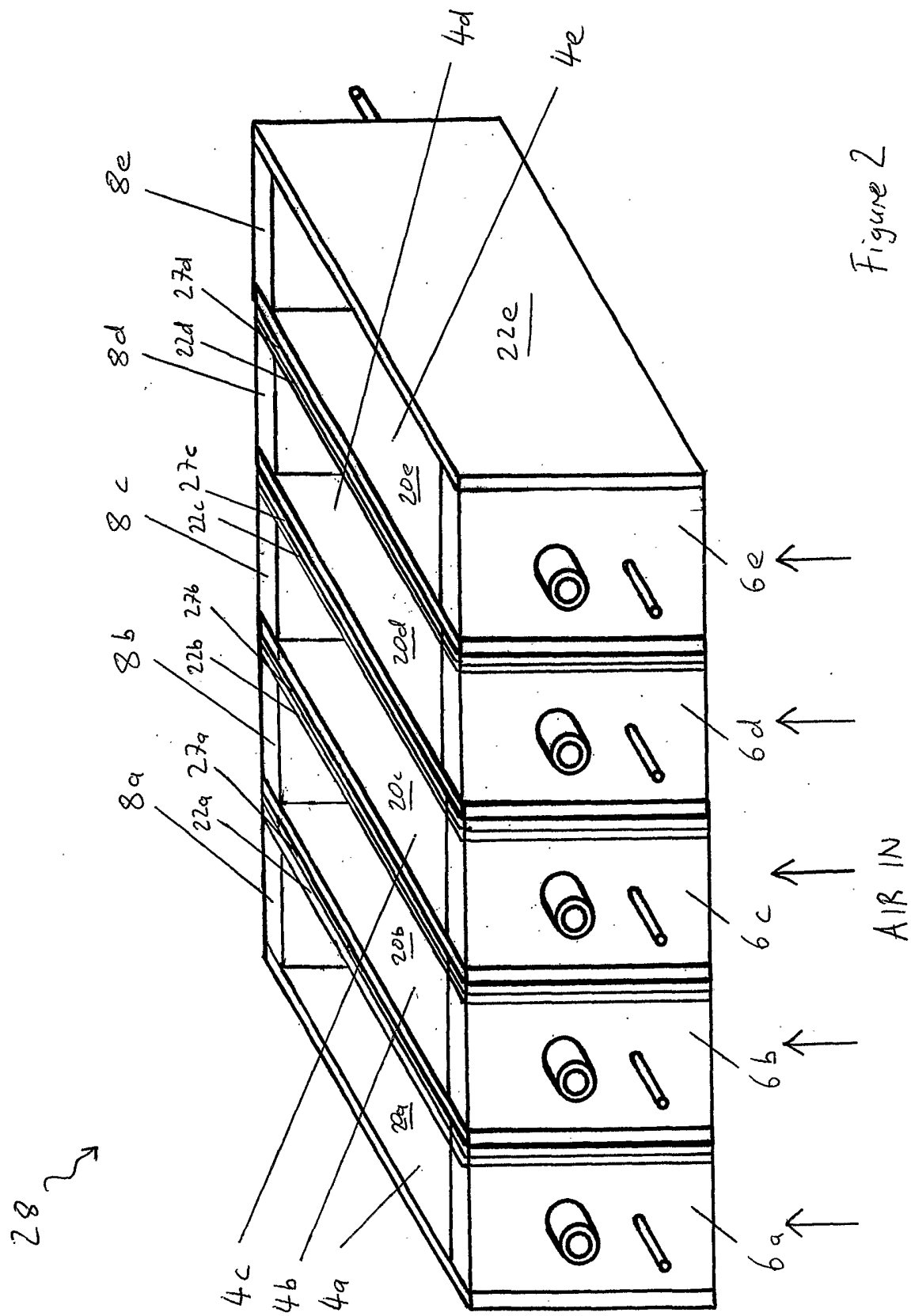



Figure 1B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20b
20c
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4e
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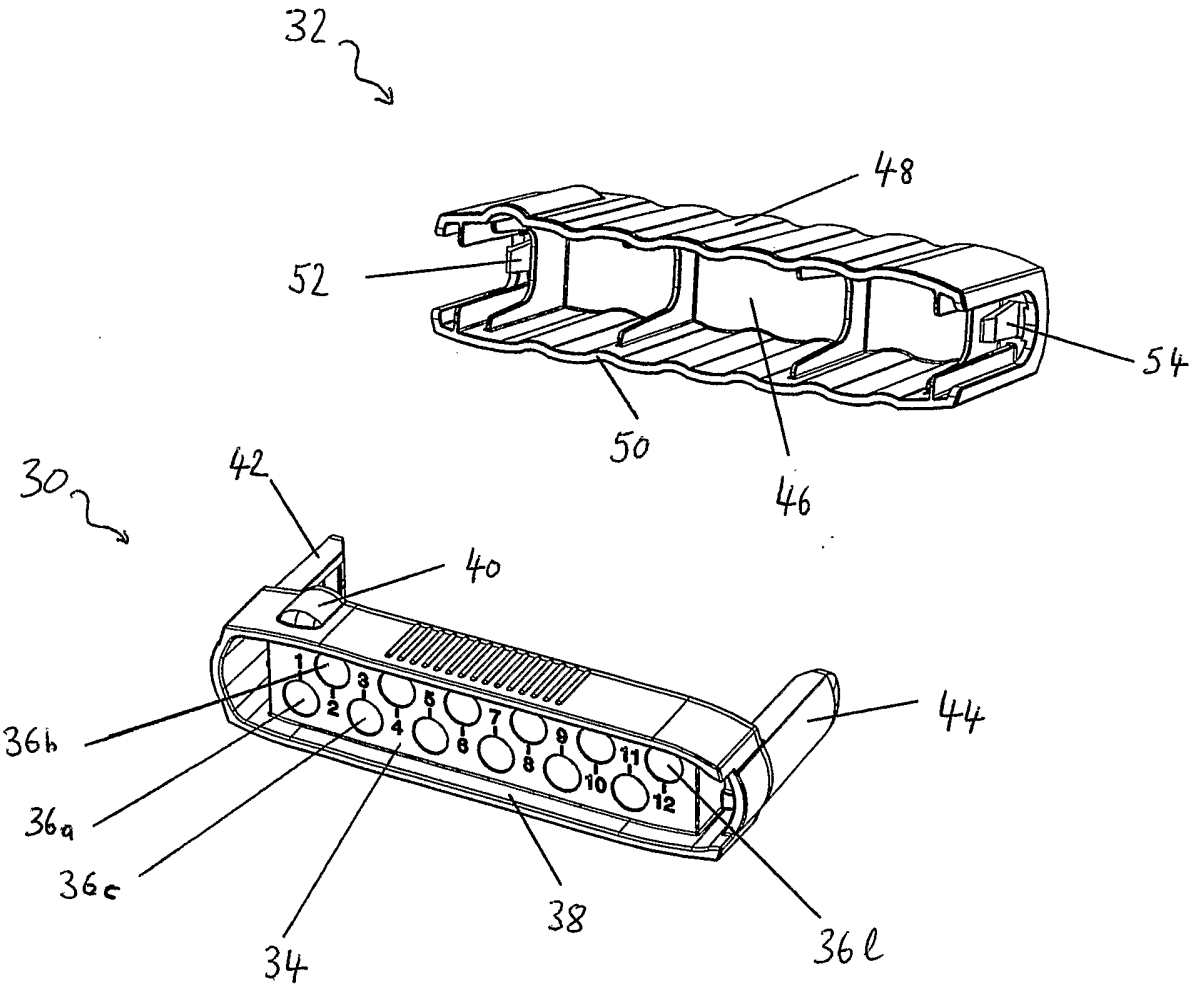
6d
6e
AIR IN

Figure 2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7
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9
10
11
12

Figure 3

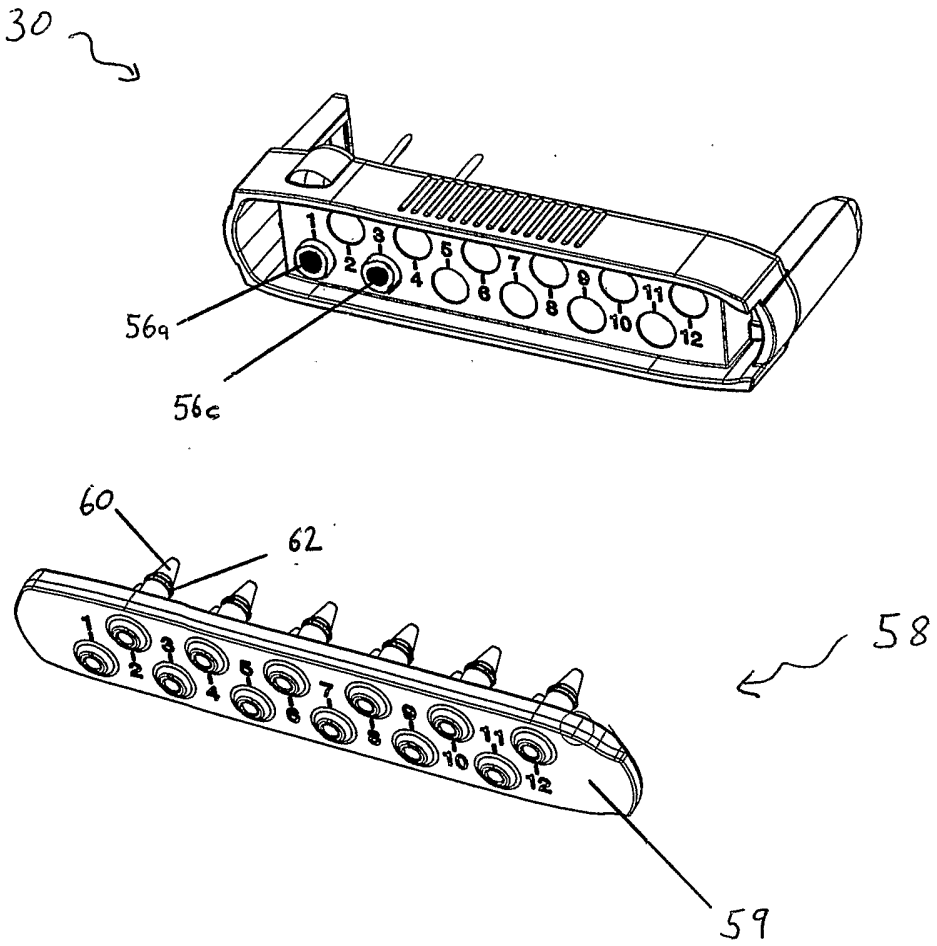
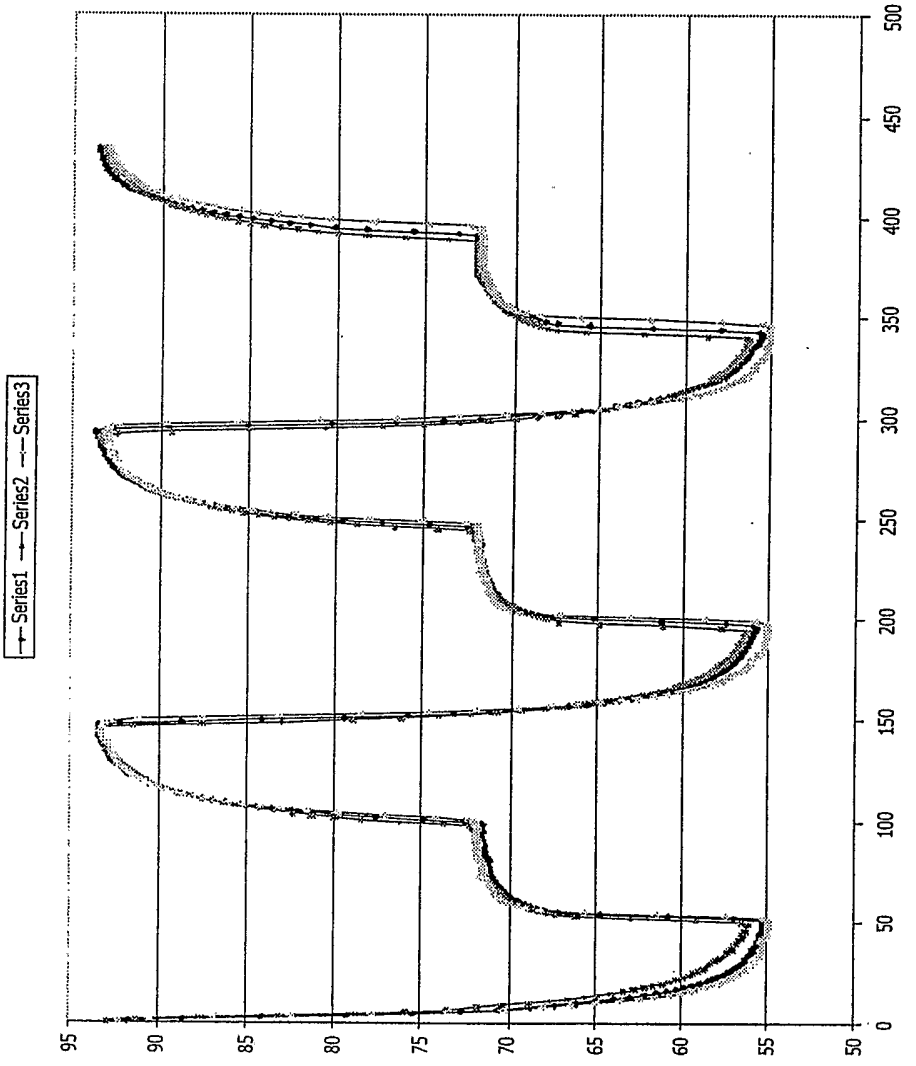



Figure 4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Series2
Series3
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90
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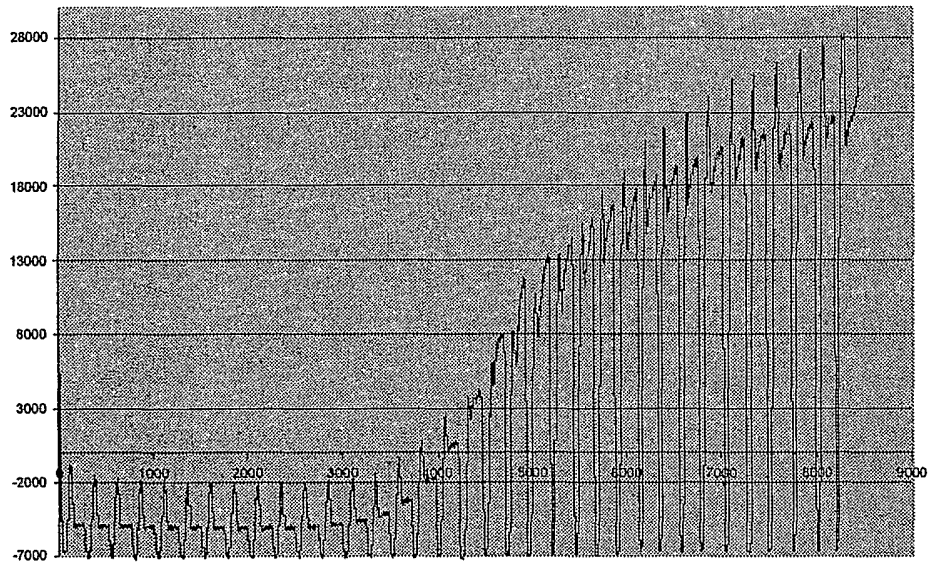
450
500

Figure 5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7000
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9000

Figure 6

# INTERNATIONAL SEARCH REPORT

PCT/GB 03/05486

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L7/00

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)
IPC 7 B01L

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 practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
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| X | the whole document | 14,15 |
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| A | US 5 455 175 A (WITTWER CARL T ET AL) 3 October 1995 (1995-10-03) the whole document | 1-15 |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 17 March 2004 | 23/03/2004 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31–70) 340–2040, Tx. 31 651 epo nl,<br>Fax: (+31–70) 340–3016 | Smith-Hewitt, L |

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