

Reaction System

The present invention relates to a method of carrying out amplification reaction, in particular, the polymerase chain
5 reaction (PCR) using a disposable unit, and to disposable units used in the method.

The controlled heating of reaction vessels in such methods is often carried out using solid block heaters which are heated
10 and cooled by various methods. Current solid block heaters are heated by electrical elements or thermoelectric devices *inter alia*. Other reaction vessels may be heated by halogen bulb/turbulent air arrangements. The vessels may be cooled by thermoelectric devices, compressor refrigerator technologies,
15 forced air or cooling fluids.

The reaction vessels, which are generally tubes or curvettes, fit into the block heater with a variety of levels of snugness. Thus, the thermal contact between the block heater and the reaction vessel varies from one design of heater to another. In
20 reactions requiring multiple temperature stages, the temperature of the block heater can be adjusted using a programmable controller for example to allow thermal cycling to be carried out using the heaters.

A disadvantage of the known block heaters arises from the lag
25 time required to allow the heating block to heat and cool to the temperatures required by the reaction. Thus, the time to complete each reaction cycle is partially determined by the thermal dynamics of the heater in addition to the rate of the reaction. For reactions involving numerous cycles and multiple
30 temperature stages, this lag time significantly affects the time taken to complete the reaction. Thermal cyclers based on such block heaters typically take around 2 hours to complete 30 reaction cycles.

For many applications of the PCR technique it is desirable to complete the sequence of cycles in the minimum possible time. In particular for example where respiratory air or fluids or foods for human and animal stock consumption are suspected of contamination rapid diagnostic methods may save considerable money if not health, even lives.

Apparatus for thermally cycling a sample are described in WO98/09728. In this apparatus the reagents are held in a disposable unit which comprises a thin planar structure so as to ensure good thermal contact with reagents contained in the unit. The units are made either of plastics materials such as polycarbonate or polypropylene, or silicon. Silicon is preferred as the thermal conductivity ensures that the reagents are heated quickly. However in order to effect a PCR reaction, where biological reagents are employed, the silicon must be coated with a biocompatible layer.

Other forms of disposable unit are described for example in EP 0723812. These include units with metal elements such as aluminium. Although such units have good thermal properties, the fact that biological reagents are in contact with the surfaces of the unit across a high surface area (i.e. there is a high surface area:volume ratio) appears to magnify any incompatibilities of the reagents, to the extent that conventional PCR reaction conditions may fail to give a reaction.

The applicants have found that surprisingly PCR reactions can be successfully effected in units which have high surface area: volume ratios and are made of relatively simple, readily available components, and that metal substrates can be used under particular PCR conditions.

According to the present invention there is provided a method of carrying out an amplification reaction, said method

comprising supplying to a well in a disposable unit (a) a sample which contains or is suspected of containing a target nucleic acid sequence (b) primers, nucleotides and enzymes required to effect said amplification reaction and (c) a buffer system, and subjecting the unit to thermal cycling conditions such that any target nucleic acid present within the sample is amplified; wherein the disposable unit comprises a thermally conducting layer and a facing layer having one or more reagent wells of up to 1000 microns in depth defined therebetween; and the reaction mixture comprises at least one of the following:

- A) a buffer system wherein the p.H. is above 8.3;
- B) a detergent; and/or
- C) a blocking agent.

Target nucleic acids include DNA and RNA.

Suitable amplification reactions include the polymerase chain reaction as mentioned above. In this case, the primers used are amplification primers and the enzymes comprise nucleic acid polymerase, in particular thermally stable DNA polymerase such as TAQ polymerase.

Suitably the wells are from 100-1000 microns in depth and preferably less than 500 microns in depth. In particular wells are from 100-500 microns in depth. Depth in this context relates to the distance between the thermally conducting layer and the facing layer.

Preferably, at least a buffer system wherein the p.H. is above 8.3 is employed.

Suitable buffer systems which allow an amplification reaction to proceed will vary depending upon the particular nature of the materials used in the construction of the disposable units and the reaction taking place. Generally speaking, the buffers

used in conventional PCR reactions have a pH of the order of 8.3 and comprise 10mM Tris HCl solution. When these conditions have been used in the disposable units described above, it may not be possible to achieve a successful
5 amplification reaction.

Buffers used in the method of the reaction are suitably at a higher pH than this. For example, the pH of the buffer is suitably from 8.5- 9.2, more suitably from 8.7-9.0 and
10 preferably at about pH 8.8 @ 25°C

The applicants have found that buffers which are at higher concentrations than standard PCR buffers are preferred. Particularly suitable buffers for use in the amplification
15 reaction of the invention comprise from 30-70mMTris HCl and preferably about 50mM Tris HCl pH 8.8 @ 25°C.

Other suitable components for the buffer solution include 1.5mM MgCl.
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Small amounts, for example from 0.01 to 0.1% v/v and preferably about 0.05% v/v, of detergents such as Tween™ or Triton™ may also be present.

25 A particular example of such a buffer system is one which comprises from 30-70mMTris HCl pH 8.8 @ 25°C.

The presence of a blocking agent such as bovine serum albumin (BSA) has been found to be advantageous, in particular where
30 the reagents undergoing reaction are directly in contact with the metal layer of the disposable unit.

Thereafter, amplification product can be detected for example, by removing the product from the well and separating it on an
35 electrophoretic gel as is known in the art. Preferably

however, reagents used in the amplification such as the primers are labelled with a fluorescent label, or a fluorescently labelled probe, able to hybridise to the target sequence under conditions that may be generated within the disposable unit.

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Where the disposable unit comprises multiple wells, each may be pre-dosed with different PCR primers as well as the DNA polymerase enzyme. This gives the possibility that a single sample may be simultaneously tested for the presence of a range of different target sequences.

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Suitably the metal used in the thermally conducting layer of the disposable unit is aluminium. The aluminium facing layer is suitably in the form of an aluminium foil. If required the foil may be coated with a plastic or other biocompatible layer but this is not required in order to effect a successful PCR reaction in accordance with the invention. A particularly suitable coating material is polystyrene or other material which allows the layer to be heat-sealed to the facing layer. This avoids the need for the presence of an adhesive. A particular example of heat-sealable polystyrene coated aluminium film is available from Advanced Biotechnologies, (Epsom UK), and is sold as Thermoseal AB-0598.

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The facing layer may be thermally conducting or thermally insulating depending upon whether it is intended to supply heat to the unit at one or both faces. Where a thermally conducting layer is required, it is suitably an aluminium layer, preferably with heat sealable coating for example of polystyrene. This allows ready manufacture of the units by heat sealing two layers together. Areas are left unsealed so as to provide one or more reagent wells between the layers as well, as channels allowing reagent materials to be introduced into the wells.

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In a preferred embodiment however, the facing layer is of a biocompatible plastics material such as polypropylene or polycarbonate, which is transparent. This allows the progress of reactions conducted in the wells to be monitored. For
5 example, where the amplified reaction utilises visible label means, such as fluorescent labels, the progress of the reaction can be monitored using a fluorescence detection device as is well known in the art. Examples of suitable fluorescent assays are described for instance in International Patent Application
10 No's PCT/GB98/03560, PCT/GB98/03563 and PCT/GB99/00504.

In a particularly preferred embodiment the unit used in the method has a composite structure comprising a spacing layer having holes and channels define the wells and channels adhered
15 between the thermally conducting layer and the facing layer. Suitably the spacing layer is of a relatively rigid biocompatible plastics material such as polycarbonate. Where an adhesive is employed to secure the layers of the composite structure, the adhesive must itself be biocompatible. An
20 example of such an adhesive is 7957MP adhesive available from 3M. Where component layers of the composite structure are heat sealable, then this may provide a preferred form of assembling the unit as the requirement for further chemicals in the vicinity of the reagent is avoided.

25 Preferably the unit contains a plurality of reagent wells, for example from 10-100 reagent wells, and generally from 30-96 wells. This form allows a plurality of different reactions to be effected at the same time. Reagents may be introduced by
30 way of one or more channels provided in the unit and open at the edge thereof.

Suitably the wells are each connected to a common reagent channel to allow ingress of sample into each well. Suitably
35 the channel is of sufficient dimensions to prevent mixing of

reagents in individual wells by convection, and furthermore to limit significant mixing as a result of diffusion effects. If required, each well can be sealable once filled, for example by mechanical deformation of one or both layers of the unit or by
5 heat sealing.

If necessary or desired spacer means such as small glass balls (Ballotini balls) may be present within the wells in order to ensure they remain sufficiently open to allow easy ingress of
10 reagents.

In general, certain reagents and in particular PCR reagent primers or probes, are introduced into the wells, suitably in dried form, prior to the construction of the unit. Thus the
15 reagents are placed or printed onto one of either the thermally conducting layer or the facing layer before the layer is adhered to the other layer or to the spacing layer where present.

20 The disposable units are suitably of a convenient size. For example, they may be of "credit card" or "chip" dimensions or they may be similar in size to a microscope slide.

Thus the units will generally be of square or rectangular shape
25 where each side is suitably from 5 to 25cm long. The thickness of the unit will depend upon the nature of the particular layers used but they will generally be as thin as possible consistent with a mechanically robust structure as this will ensure that reagents are heated in as rapid and as even a
30 manner as possible.

Generally however, the thermally conducting layer and any thermally conducting facing layer will be of the order of from 5-25 microns thick. Thermally insulating spacing layers may be
35 thicker, for example from 100-500 microns thick. Spacing

layers will be sufficiently thick to ensure that the well dimension is of the order of from 100-1000 microns, preferably from 100-500microns. Other spacing means, such as Ballotini balls, where used, will be suitably dimensioned to ensure this level of distance between the conducting layer and the facing layer in the wells.

Preferably the opening into wells within the unit is by way of a common channel which has a single opening in order to simplify the filling operation and to minimise the risk of contamination. In order to fill such a unit with a liquid sample, air must be expelled. This may be done by means of a pump arrangement or by filling the unit in a vacuum chamber. The access channel of the unit is placed in contact with a liquid sample which will generally include PCR buffers, within a vacuum chamber. The chamber is first evacuated to eliminate air from the unit. Subsequent return to pressure forces liquid into the wells in the unit.

This arrangement of disposable unit forms a further aspect of the invention. Thus in a further embodiment, the invention provides a disposable unit for conducting a thermal cycling reaction, said unit comprising a thermally conducting layer and a facing layer having a plurality of reagent wells defined therebetween, characterised in that all the wells are fed by a common channel which includes a single opening to the outside of the unit.

Suitably such units may include some or all the other preferred features described above. In particular the wells are predosed with dried reagents, such as PCR reagent primers or probes. In addition thermally conducting layer is suitably a metal layer.

In a further embodiment, the invention provides a method of filling a disposable unit as described above with a liquid,

said method comprising using air pressure to force the liquid into the unit. This may be effected by placing the unit and said liquid in a vacuum chamber, reducing pressure in said chamber such that gas is evacuated from the disposable unit, 5 immersing at least the opening of said unit in said liquid, and increasing pressure in said chamber such that liquid is forced to enter the unit through the opening.

Preferably, the opening is immersed in said liquid before the 10 pressure in the chamber is reduced.

Suitable vacuum chambers include vacuum ovens as are known in the art.

15 The disposable units described above can be used in a variety of apparatus adapted for thermal cycling reactions including that described in WO98/09728.

In a particularly preferred embodiment however, the method is 20 effected in apparatus which comprises a plurality of heating blocks and conveyor means for holding and moving disposable units between the blocks. Suitably there are sufficient blocks to effect different stages of an amplification reaction. For example, a typical PCR reaction involves a cycling process of 25 three basic steps.

Denaturation : A mixture containing the PCR reagents (including the nucleic acid to be copied, the individual nucleotide bases (A,T,G,C), suitable primers and polymerase enzyme) are heated to a predetermined temperature to separate the two strands of 30 the target nucleic acid.

Annealing : The mixture is then cooled to another predetermined temperature and the primers locate their complementary sequences on the nucleic acid strands and bind to them.

Extension : The mixture is heated again to a further predetermined temperature. The polymerase enzyme (acting as a catalyst) joins the individual nucleotide bases to the end of the primer to form a new strand of nucleic acid which is complementary to the sequence of the target nucleic acid, the two strands being bound together.

Typical denaturation temperatures are of the order of 95°C, typical annealing temperatures are of the order of 55°C and extension temperatures of 72°C are generally of the correct order.

In a preferred apparatus for use in the method of the invention, at least two and preferably three heating blocks are provided, each of which is under the control of an automatic temperature control means. In use, one block is maintained at the denaturation temperature, one block is maintained at the annealing temperature and one block is maintained at the desired extension temperature. The disposable unit is then transferred sequentially between the blocks using the conveyor means, such as a conveyor belt, and held in the vicinity of each of the said blocks for a sufficient period of time to allow the unit to reach the temperature of the block and to allow the relevant stage of the amplification reaction to take place. The conveyor means suitably comprises a timing belt attached to a stepper motor.

Each heating block can be segregated such that individual wells or groups of wells within the disposable unit reach different temperatures in some or all of the reaction stages. For example, the annealing block could be segregated into four zones to allow four different annealing temperatures to be reached in different wells in the disposable unit. This may be required to ensure the specificity of four different specific amplification reactions.

If necessary, actuators such as solenoids, may be provided above each block and arranged to clamp the disposable unit against the block when it is arranged above it so as to ensure good thermal contact.

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Suitably the actuators themselves may comprise heating elements, which are maintained at similar temperatures to the blocks. These can then contribute to the heating effect to ensure that the desired reaction temperature can be reached within the unit as rapidly as possible. This may be particularly useful where the facing layer of the disposable unit is a thermally conducting layer such as an aluminium layer.

15 Operation of the conveyor means, the heating blocks, the actuators and the heating elements are controlled automatically by a computer operating a suitable algorithm to effect the desired amplification reaction.

20 An alternative form of heating apparatus may comprise an electrically conducting polymer, which may be integral with or arranged in close proximity to the disposable unit. Such apparatus is described and claimed in PCT/GB97/03187.

25 In a particularly preferred embodiment, the apparatus used in the method further comprises means to detect the presence of labelled reagents within the disposable unit. This may comprise a fluorescence detector device as mentioned above. Where the facing layer of the disposable unit is of a transparent material, the fluorescence detector device can be used to detect signal generated within a well either at the end of or at any stage during the amplification reaction. Such a system may be particularly useful in connection with assays such as the TAQMAN™ assay, where continuous monitoring of the

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signal from a dual labelled probe during a PCR reaction provides the basis for quantitation of the target sequence.

The detector device is suitably arranged such that the conveyor
5 means passes the disposable unit before it at the desired stage or stages during the amplification reaction.

Amplification reactions as described above are suitably carried out rapidly, for example in less than 20 minutes. This may be
10 achieved by holding the reagents at the temperatures required for the various for about 30 seconds. This means that the results of the reaction can be ascertained early and also that the effects of diffusion of reagents between wells where there is a common channel are minimised or eliminated.

15 In a particular embodiment, the invention provides method of carrying out an amplification reaction, said method comprising supplying to a well in a disposable unit as described above (a) a sample which contains or is suspected of containing a target
20 nucleic acid sequence (b) primers and enzymes required to effect said amplification reaction and (c) a buffer system which allows the amplification reaction to be carried out in said unit; subjecting the unit to thermal cycling conditions such that any target nucleic acid present within the sample is
25 amplified.

Preferred variants including buffer systems, disposable units etc. are as set out above. In particular, said disposable unit comprises a thermally conducting layer and a facing layer
30 having one or more reagent wells defined therebetween, characterised in that said thermally conducting layer comprises a metal.

The invention will now be particularly described by way of example with reference to the accompanying diagrammatic drawings in which:

- 5 Figure 1 shows an embodiment of a disposable unit useful in the method of the invention;

Figure 2 is an expanded section on line X-X of Figure 1;

- 10 Figure 3 shows an alternative embodiment of the disposable unit useful in the method of the invention;

Figure 4 is a schematic diagram of apparatus used to fill a disposable unit.

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The following Example illustrates the invention.

- The disposable unit 1 illustrated in Figure 1 comprises a "credit card" size unit having a thin (approximately 10-20 μ m) backing layer 2 of aluminium foil (Figure 2). A spacing layer 3 of polycarbonate approximately 175-250 μ thick is adhered to the backing layer 2 by means of an adhesive layer 4. Holes 5 and a channel 6 interconnected with the holes 5, is provided in the spacing layer 3. A facing layer 7, also of polycarbonate and of the order to 175 μ m thick is adhered to the spacing layer 3 by a further adhesive layer 8.
- 20
- 25

- Dried reagents (not shown) such as PCR reagents as described above may be applied to the backing layer 2 or the facing layer 7 prior to assembly by the adhesive layers. These reagents are applied such that they will be coincident with holes 5 spacing layer 3.
- 30

- Once assembled, the holes 5 define reagent wells containing the pre-dried reagents.
- 35

In the embodiment of Figure 3, both the backing layer 3 and the facing layer 7 comprise a heat sealable aluminium foil, in particular Thermoseal, which comprises a 20µm thick aluminium layer coating with an approximately 5µm thick polystyrene coating thereon. By selectively heat sealing the layers together, wells 10 and an interconnecting channel 11 can be defined.

Spacing within the wells is achieved in this instance by the presence of glass Ballotini balls 12, suitably ranging in size from 210 to 325µm diameter.

Again, dried reagents such as PCR reagents appropriate for use in the method of the invention are suitably applied to either the backing layer 3 or the facing layer 7 prior to heat sealing, and arranged such that in the final unit, they are present within the wells 10.

The arrangement illustrated in Figure 4 shows one system for filling the units. This system comprises a vacuum oven 13 attached to a vacuum pump 14 which is controlled by a regulator 15. A regulator valve 16 is provided in the system so as to allow the system to be opened to atmosphere. A disposable unit 1, pre-dosed with dried PCR reagents, is placed in the oven within a container 17 and arranged such that the open end of the channel is in contact with a liquid 18 comprising the sample under test and buffers etc. required for the PCR reaction.

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The vacuum pump 14 is then operated to evacuate the oven 13. Air in the wells 5 and channel 6 in the disposable unit 1 is bubbled through the liquid 18. Once the vacuum has been established, the pressure within the oven 13 is allowed to

increase by operation of the valve 16, whereupon liquid 18 is forced into the channel 6 and wells 5 of the unit 1.

The filled unit is then removed from the oven and the open end
5 of the channel 6 sealed for example by heat sealing if appropriate or by addition of an adhesive such as Araldite™.

This unit is then subjected to thermal cycling such that PCR
amplification reactions take place in each well provided the
10 sample includes nucleic acid which hybridises to the primers present in the well.

Example 1

Materials used in this experiment were magnesium Chloride
15 (Product No M-1028), Bovine Serum Albumin (Product No B-8667), Glycerol (Product No G-5516), Trizma® pre-set crystals pH 8.8 (Product No T-5753), Tween®20 (Product No P-2690), HPLC Mega Ohm water (Product No 27,073-3) and Ammonium Sulphate (Product No 7783-20-2), obtained from Sigma Chemicals, Fancy Road,
20 Poole, Dorset, UK. Taq DNA polymerase 5 units/μl, and PCR dNTP's nucleotides were obtained from Boehringer Mannheim UK (Diagnostics & Biochemicals) Limited, Bell Lane, Lewes, East Sussex BN7 1LG, UK). Custom oligonucleotide primers (HPLC Grade) were obtained from Cruachem Ltd, Todd Campus, West of
25 Scotland Science Park, Acre Road, Glasgow G20 0UA, UK.

The target DNA was an engineered internal control construct, pYP100ML, containing PCR primer sites for the anticoagulase gene of *Yersinia pestis*. The primer sequences were YPPA155
30 (dATGACGCAGAAACAGGAAGAAAGATCAGCC) and YPF229R (dGGTCAGAAATGAGTATGGATCCCAGGATAT). These primers amplify a 104bp amplicon.

Reagents were prepared using the formulations in Tables 1. The buffers had four different adjuncts added, resulting in 16 buffer formulations (Table 2).

- 5 PCR was performed with one of the buffer combinations, 200 μ M dNTP's (each), 1 μ M primers, and 0.04U/ μ l Taq DNA polymerase. 10pg/ μ l of pYP100ML construct was used as DNA template.

- The apparatus for filling the disposable units consisted of an
10 Edwards Speedvac II pump connected to a vacuum oven.

- PCR reagents (~250 μ l volume) were loaded into the groove of the filling tool and the disposable unit set in place. The unit and filling tool were placed into a vacuum oven and a vacuum
15 was drawn. The pump was operated in accordance with the manufacturer's instructions. Once a vacuum of ~20mbar was reached, the pump was switched off. Once the pressure was equilibrated at atmospheric pressure, the disposable unit assembly was removed. The channels in the disposable units
20 contained the PCR reagents. The opening of the credit card was sealed with a PCR compatible adhesive (Araldite®) was allowed to cure on ice for ~1hr.

- Testing of the disposable units was carried out on the Perkin
25 Elmer 9700 machine using the following temperature profile: denature at 97°C for 20 seconds, annealing at 50°C for 20 seconds, and extension at 75°C for 20 seconds. The 9700 block was flooded with oil to ensure good thermal contact between the block and credit card. Control PCR reaction mixtures were also
30 run on this machine using the above parameters.

Testing was also carried out on a prototype Thermal Cycling Instrument using the following reaction parameters: denature at

98°C for 10 seconds, annealing at 50°C for 10 seconds, and extension at 77°C for 10 seconds.

Positive and negative (no template) controls were performed in
5 MicroAmp® reaction vessels and thermocycled in the Perkin
Elmer 9700 PCR instrument.

The sample was carefully extracted from the credit card by means of a pipette tip and analysed by conventional agarose gel
10 electrophoresis for signs of successful DNA amplification. The
PCR products were run on a 2% (w/v) agarose in 1X T.A.E.
buffer. Ethidium bromide was added to the gel at a final
concentration of 0.5µg/ml. Electrophoresis was performed in 1X
T.A.E. buffer and allowed to run for ~30-40 minutes at 100
15 volts. Following electrophoresis, bands on the gel were
visualised using ultraviolet light and images recorded using a
Bio/Gene gel documentation system.

The YPPA155/YPP229R primer pair and pYP100ML construct was used
20 to study the biocompatibility of two types of disposable unit as
a platform for PCR.

The first was a unit where both the thermally conducting layer and the facing layer were of Thermo-seal aluminium which had
25 been heat sealed together and contained Ballotini balls as
spacers. The second unit was a composite unit, comprising an
aluminium foil layer as the thermally conducting layer, a
transparent polycarbonate layer as the facing layer and a
polycarbonate spacing layer (175µm thick). Layers were adhered
30 together using 7957MP adhesive.

The units were evaluated for PCR compatibility as well as structural integrity and retention of volume during thermal cycling.

All the chemistry PCR formulations were tested on a block thermal cycler in a tube PCR and were shown to be effective when analysed using the technique of agarose gel electrophoresis.

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Work then commenced on testing the PCR formulations in the disposable units of the invention. The compositions which gave positive results are indicated in Table 3 hereinafter.

- 10 Particularly rapid PCR reactions of approximately 19 minutes were achieved using apparatus of the invention comprising 3 heating blocks as described above.

The study demonstrated the using the disposable units of the
15 invention as a PCR platform.

Table 1: Buffer Composition. Final 1X composition

Buffer	Composition
1	50mM Tris.HCl pH8.8 1.5mM MgCl ₂
2	50mM Tris.HCl pH8.8 1.5mM MgCl ₂ 20mM (NH ₄) ₂ SO ₄
3	75mM Tris.HCl pH8.8 1.5mM MgCl ₂
4	75mM Tris.HCl pH8.8 1.5mM MgCl ₂ 20mM (NH ₄) ₂ SO ₄

Table 2: Adjuncts added to Buffers. Final 1X composition

	Adjuncts
A	0.05% (v/v) TWEEN + 250ng/ μ l BSA
B	0.05% (v/v) TWEEN
C	8% (v/v) Glycerol + 250ng/ μ l BSA
D	Native (No adjuncts added)

Table 3: A summary of the results obtained on the affect of
 5 using disposable units of the invention as a platform for PCR

Disposable unit	Materials exposed to PCR solution	Successful chemistry composition
Thermo-seal aluminium	Polycarbonate, Polystyrene, Glass	Buffer 1 Adjunct B
		Buffer 1 Adjunct A
		Buffer 1 Adjunct B
		Buffer 2 Adjunct A
		Buffer 4 Adjunct A
		Buffer 4 Adjunct B
Composite	Polycarbonate, Aluminium, 7957MP Adhesive	Buffer 2 Adjunct A

Example 2

10 A range of materials including aluminium and Thermo-seal foil
 AB0598 with a polystyrene coating were tested for possible use
 in the development of a disposable unit for PCR. These
 were tested under normal PCR conditions and in the presence of
 a blocking agent (BSA) to determine their compatibility with
 15 the reaction.

About 25 pieces, 5mm x 5mm square (approx), of each material were cut from sheets supplied. These were put into 1.5 ml Eppendorf tubes with 1 ml 10% Tween 20 in deionised water. The tubes were vortexed and placed at 70°C for 1 - 2 hours.

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The pieces were recovered by filtration through 1 layer of blue roll, placed in about 10 ml deionised water in a 25 ml sample bottle and shaken. This filtration and wash step was done 3 times.

- 10 Pieces of material were then placed in 1.5 ml Eppendorf tubes and stored, refrigerated, until used in a PCR reaction.

Washed samples of the materials were placed in Perkin Elmer PCR reaction tubes with various PCR mix as follows:

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PCR Reagents

10mM Tris.HCl pH 8.3

50mM KCl

2mM or 5mM MgCl₂

- 20 0.2mM each dNTP

1µM each primer

1.25u Taq DNA polymerase

0 or 0.025% Bovine Serum Albumen (BSA)

0 or 0.5ng *E.coli* DNA

- 25 In a volume of 50µl.

The primers used delineate a 663 base section of the *E.coli* Aro A gene. The left primer is a 22mer and the right one a 21mer.

The PCR thermal cycle was:

- 30 94°C x 5 min (94°C x 30s, 55°C x 30s, 72°C x 1min)₃₀ 72°C x 7 min, 4°C hold.

Either 1 or 2 pieces of each material were added to the reaction. Control reactions without test material and without

DNA template were run each day. Amplicon was detected as bands on a gel. The results are summarised in Table 4.

Table 4

Material	PCR Mix			
	2mM MgCl ₂	5mM MgCl ₂	2mM MgCl ₂ + BSA	5mM MgCl ₂ + BSA
1 piece Aluminium foil (unwashed)	-	-	+	+
1 piece Aluminium foil (washed in Tween)	-	-	++	++
1 piece Thermo-seal foil AB-0598	-	-	++	++
2 pieces Aluminium foil (unwashed)	-	-	+	+
2 pieces Aluminium foil (washed in Tween)	-	-	+	++
2 pieces Thermo-seal foil AB-0598	-	-	-	++

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where - indicates that no band was seen

+ indicates the presence of a band

++ indicates the presence of a brighter band.

- 10 The results show that BSA increased the compatability of the aluminium based materials (as well as many others - results not shown).

Claims

1. A method of carrying out an amplification reaction, said method comprising supplying to a well in a disposable unit (a)
5 a sample which contains or is suspected of containing a target nucleic acid sequence (b) primers, nucleotides and enzymes required to effect said amplification reaction and (c) a buffer system, and subjecting the unit to thermal cycling conditions such that any target nucleic acid present within the sample is
10 amplified; wherein the disposable unit comprises a thermally conducting layer and a facing layer having one or more reagent wells of up to 1000 microns in depth defined therebetween; and the reaction mixture comprises at least one of the following:
A) a buffer system wherein the p.H. is above 8.3;
15 B) a detergent; and/or
C) a blocking agent.
2. A method according to claim 1 wherein the p.H. of the buffer system is above 8.3.
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3. A method according to claim 2 wherein the p.H. of the buffer system is from 8.7-9.0
4. A method according to any one of the preceding claims
25 wherein the buffer system comprises from 30-70mM Tris HCl.
5. A method according to claim 5 wherein the buffer system comprises about 50mM Tris HCl pH 8.8 @ 25°C.
- 30 6. A method according to any one of the preceding claims wherein the reaction mixture further comprises from 0.01 to 0.1% v/v detergents.
7. A method according to claim 6 wherein the said detergent
35 comprises Tween TM or Triton TM.

8. A method according to anyone of the preceding claims wherein the reaction is effected in the presence of a blocking agent which comprises bovine serum albumin (BSA).

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9. A method according to any one of the preceding claims wherein the thermally conducting layer of the disposable unit is metal.

10 10. A method according to any one of the preceding claims wherein the thermally conducting metal layer of the disposable unit is aluminium.

11. A method according to any one of the preceding claims
15 wherein the thermally conducting metal layer is coated with a plastic or other biocompatible layer.

12. A method according to claim 11 wherein the biocompatible layer is polystyrene.

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13. A method according to any one of the preceding claims wherein the thermally conducting layer and the facing layer of the disposable unit are heat sealed together.

25 14. A method according to any one of the preceding claims wherein the facing layer of the disposable unit comprises a thermally conducting layer.

15. A method according to any one of the preceding claims
30 wherein the facing layer of the disposable unit is of a transparent biocompatible plastics material.

16. A method according to any one of the preceding claims which further comprises a spacing layer having holes and channels to

define reagent wells and channels adhered between the thermally conducting layer and the facing layer.

17. A method according to claim 16 wherein the layers are
5 adhered together by means of a biocompatible adhesive.

18. A method according to any one of the preceding claims wherein spacer means are provided within each reagent well.

10 19. A method according to any one of the preceding claims wherein the wells are pre-dosed with dried reagents.

20. A method according to claim 15 wherein the dried reagents are PCR reagent primers or probes.

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21. A method according to any one of the preceding claims wherein the disposable unit contains a plurality of wells and each well is fed by a common channel which has a single opening to the outside of the unit.

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22. A method according to any one of the preceding claims wherein the disposable unit is placed in apparatus comprising at least two heating blocks, each of which is under the control of an automatic temperature control means, and conveyor means
25 for holding and transferring a disposable unit according to any one of the preceding claims sequentially between the blocks.

23. A method according to claim 22 wherein the apparatus further comprises an actuator above each block and arranged to
30 clamp the disposable unit against the respective block.

24. A method according to claim 22 or claim 23 wherein the heating block is segregated and each segment is held at a different temperature.

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25. A method of carrying out an amplification reaction, said method comprising supplying to a reagent well in a disposable unit, a reaction mixture comprising: (a) a sample which contains or is suspected of containing a target nucleic acid
5 sequence; (b) primers, nucleotides and enzymes required to effect said amplification reaction; and (c) a buffer system, wherein the pH of said buffer system is from 8.7-9.0, and subjecting the disposable unit to thermal cycling conditions such that any target nucleic acid present within the sample is
10 amplified; wherein the disposable unit comprises a thermally conducting layer and a facing layer having one or more reagent wells up to 1000 microns in depth defined therebetween, and wherein said reaction mixture further comprises a detergent and/or a blocking agent.

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26. A method of carrying out an amplification reaction, said method comprising supplying to a reagent well in a disposable unit, a reaction mixture comprising: (a) a sample which contains or is suspected of containing a target nucleic acid
20 sequence; (b) primers, nucleotides and enzymes required to effect said amplification reaction; and (c) a buffer system, wherein the pH of said buffer system is at or above 8.8, and subjecting the disposable unit to thermal cycling conditions such that any target nucleic acid present within the sample is
25 amplified; wherein the disposable unit comprises a thermally conducting layer and a facing layer having one or more reagent wells up to 1000 microns in depth defined therebetween, and wherein said reaction mixture further comprises a detergent and/or a blocking agent.

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27. The method according to claim 25 or 26, wherein the buffer system comprises from 30-70 mM Tris HCl.

28. The method according to claim 27, wherein the buffer
35 system comprises about 50 mM Tris HCl, pH 8.8 at 25°C.

29. The method according to any one of claims 25 to 28 wherein the reaction mixture further comprises from 0.01 to 0.1% v/v detergents.

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30. The method according to any one of claims 25 to 29 wherein the reaction is effected in the presence of a blocking agent which comprises bovine serum albumin (BSA).

10 31. The method according to any one of claims 25 to 30 wherein the thermally conducting layer of the disposable unit is metal.

32. The method according to claim 31 wherein the thermally
15 conducting metal layer of the disposable unit is aluminium.

33. The method according to claim 31 or claim 32 wherein the thermally conducting metal layer is coated with a plastic or other biocompatible layer.

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34. The method according to claim 33 wherein the biocompatible layer is polystyrene.

35. The method according to any one of claims 31 to 34
25 wherein the thermally conducting layer and the facing layer of the disposable unit are heat-sealed together.

36. The method according to any one of claims 25 to 35 wherein the facing layer of the disposable unit comprises a
30 thermally conducting layer.

37. The method according to any one of claims 25 to 36 wherein the facing layer of the disposable unit is of a transparent biocompatible plastics material.

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38. The method according to any one of claims 25 to 37 wherein the disposable unit further comprises a spacing layer having holes and channels to define reagent wells and channels adhered between the thermally conducting layer and the facing layer.

39. The method according to claim 39 wherein the layers are adhered together by means of a biocompatible adhesive and the reaction is effected in the presence of a blocking agent which comprises bovine serum albumin.

40. The method according to any one of claims 25 to 39 wherein the spacer means are provided within each reagent well.

41. The method according to any one of claims 25 to 40 wherein the reagent wells are pre-dosed with dried reagents.

42. The method according to claim 41 wherein the dried reagents are PCR reagent primers or probes.

43. The method according to any one of claims 25 to 42 wherein the disposable unit contains a plurality of reagent wells and each reagent well is fed by a common channel which has a single opening to the outside of the unit.

44. The method according to any one of claims 25 to 43 wherein the disposable unit is placed in apparatus comprising at least two heating blocks, each of which is under the control of an automatic temperature control means, and conveyor means for holding and transferring a disposable unit sequentially between the blocks.

45. The method according to claim 44 wherein the apparatus further comprises an actuator above each block and arranged to clamp the disposable unit against the respective block.

46. The method according to claim 45 wherein at least one of the heating blocks is segregated and each segment is held at a different temperature.
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47. The method according any one of claims 25 to 46 wherein the disposable unit is integral with or arranged in close proximity to an electrically conducting polymer.
- 10 48. The method according to any one of claims 25 to 47 wherein the presence of labelled reagents within the disposable unit is monitored.
49. A method according to any one of claims 25 to 43 wherein
- 15 the disposable unit is integral with or arranged in close proximity to an electrically conducting polymer.
50. A method according to any one of claims 25 to 51 wherein the presence of labelled reagents within the disposable unit is
- 20 monitored.
51. A method of filling a disposable unit for conducting thermal cycling reactions with a liquid, comprising:
- 25 using air pressure to force liquid into the disposable unit
- wherein the disposable unit for conducting thermal cycling reactions comprises a thermally conducting layer and a facing layer having a plurality of reagent wells defined therebetween; wherein all the reagent wells are
- 30 fed by a common channel which includes a single opening to the outside of the disposable unit;
- and wherein the method comprises placing the disposable unit and said liquid in a vacuum chamber, reducing pressure in said vacuum chamber such that air is
- 35 evacuated from the disposable unit, immersing at least

the single opening of said unit in said liquid, and increasing pressure in said vacuum chamber such that liquid is forced to enter the disposable unit through the single opening.

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52. The method according to claim 51 wherein the single opening is immersed in said liquid before the pressure in the vacuum chamber is reduced.

10 53. A kit for conducting a polymerase chain reaction, said kit comprising a buffer system comprising a buffer of p.H. in excess of 8.3, and at least one disposable unit comprising a thermally conducting layer and a facing layer having one or more reagent wells of up to 1000 microns depth defined
15 therebetween.

54. A kit according to claim 53 wherein the thermally conducting layer of the disposable unit is a metal layer.

20 55. A disposable unit for conducting a thermal cycling reaction, said unit comprising a thermally conducting layer and a facing layer having a plurality of reagent wells defined therebetween, characterised in that all the wells are fed by a common channel which includes a single opening to the outside
25 of the unit.

56. A disposable unit according to claim 55 wherein the wells are predosed with dried reagents.

30 57. A disposable unit according to claim 56 wherein the dried reagents are PCR reagent primers or probes.

58. A disposable unit according to any one of claims 55 to 57 wherein said thermally conducting layer is a metal layer.

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59. A method of filling a disposable unit according to any one of claims 55 to 58 with a liquid, said method comprising using air pressure to force liquid into said unit.

5 60. A method according to claim 59 which comprises placing the unit and said liquid in a vacuum chamber, reducing pressure in said chamber such that air is evacuated from the disposable unit, immersing at least the opening of said unit in said liquid, and increasing pressure in said chamber such that
10 liquid is forced to enter the unit through the opening.

61. A method according to claim 60 wherein the opening is immersed in said liquid before the pressure in the chamber is reduced.