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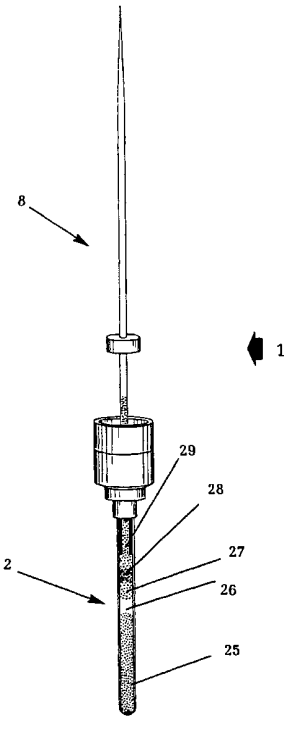
(54) Title: DIRECT ASPIRATION-REACTION AND INJECTION DEVICE AND METHODS OF USE



(57) Abstract: A device (8) comprising a chamber (25) having a first open end (3) and a second closed end (4), an elongate member (11) having first and second open ends (9, 10), and sealing means providing a seal between the elongate member (11) when received in the chamber (25). The second end (10) of the elongate member is slidably movable from a first position within the chamber (25) to a second position within the chamber (25) causing a change in pressure within the chamber (25). The device enables sample collection and analysis to be performed in a single chamber (25). Devices and methods including wax and reagent compositions within the chamber (25) are disclosed.

DIRECT ASPIRATION-REACTION AND INJECTION DEVICE AND METHODS OF USE

Field of the Invention:

The present invention relates to a device which is suitable for aspiration and analysis of biological samples, and for injection of samples into biological materials. The present invention also relates to the use of this device in methods for detecting target molecules in samples.

Background of the Invention:

Clinical diagnostic assays typically involve the collection of biological samples and subsequent analysis of those samples by assay procedures. In many cases, the sample collection and subsequent analysis are conducted in different areas of the laboratory, or outside the laboratory, thereby necessitating transport and/or transfer of biological samples and assay reagents. The handling of these samples gives rise to increased risk of contamination by other biological materials.

The risk of contamination is of particular concern when the process includes nucleic acid amplification reactions such as the polymerase chain reaction (PCR), which is capable of multiplying a single strand of nucleic acid (target nucleic acid) into millions of copies (amplicons). Although PCR technology is of enormous potential utility in the clinical diagnostic laboratory, nucleic acid amplification reactions can easily become contaminated with foreign nucleic acid molecules. For example, contamination may occur during the sampling procedure. In addition, the sample may become contaminated with the amplification products (amplicons) of previous amplification reactions, where opening of the reaction chamber, and the utilization of standard pipetting techniques, creates aerosols. Such contaminants may be amplified in the PCR reaction, leading to a false positive indication of the substrate to be detected and ultimately to incorrect diagnosis.

Due the problems discussed above, many reactions, particularly highly sensitive PCR assays, are slow and require considerable operator skill in order to ensure that reliable results are routinely obtained.

Accordingly, products and procedures which simplify sample collection and analysis and which reduce the risk of contamination are highly desirable.

Summary of the Invention:

The present inventors have now developed a device which reduces problems associated with the multiple steps involved in clinical diagnostic assays, allowing accurate results to be obtained more reliably. In particular, the device enables the sample collection and analysis to be performed in a single chamber, thereby reducing the risk of contamination.

In a first aspect, the present invention provides a device comprising a chamber having a first open end and a second closed end and an inner and an outer surface, an elongate member having first and second open ends and having an inner and an outer surface, and sealing means providing a seal between the elongate member and the chamber, the second end of the elongate member being slidably movable from a first position within the chamber to a second position within the chamber, the movement of the second end of the elongate member from the first position to the second position causing a change in the pressure within the chamber.

In a preferred embodiment of the first aspect, the chamber is elongate.

In a further preferred embodiment of the present invention, the chamber allows transmission of electromagnetic radiation. The second end of the elongate chamber may comprises a lens for transmitting electromagnetic radiation. Preferably, the electromagnetic radiation is light of a wave length between 400 to 800 nm. More preferably, the electromagnetic radiation is fluorescence.

In a further preferred embodiment, the chamber is cylindrical. Preferably, the chamber has an outer diameter of up to 2.8 mm, and/or an inner diameter of up to 2.5 mm. Preferably, the chamber has a length of between 20 to 40 mm.

Preferably, the elongate member has an outer diameter of up to 2.5 mm and an inner diameter of between 0.2 mm and 1.2 mm.

In a further preferred embodiment, the first end of the elongate member comprises a formed tip. Preferably, the tip is capable of piercing biological tissue. In a preferred embodiment, the tip is in the order of 1μm,

but can extend up to 200μm or more in diameter. Preferably the diameter of the tip is between 20 and40 μm.

In yet another embodiment, the chamber and elongate member are formed of the same material. Preferably, this material is glass or plastic. In a preferred embodiment, however, the elongate chamber is made of plastic and the elongated member is made of glass. Suitable plastics include, but are not limited to, polyethylene terephthalate, polypropylene and polycarbonate.

In a preferred embodiment, the sealing means comprises a sleeve located on the outer surface of the elongate member proximate the second end. Preferably, the sleeve co-operates with the inner surface of the chamber to form a seal. The sleeve may be of any suitable length. For example, the sleeve may be between 10 and 80 mm in length. It will be appreciated, however, that a longer sleeve may be desirable as it will impart additional rigidity to the elongate member. The sleeve may co-operate with the inner surface of the chamber along the entire length of the sleeve. It is preferred, however, that the sleeve comprises a flared region of about 0.2 to 2.0 mm in length which co-operates with the inner surface of the chamber to form the seal.

In a preferred embodiment, the chamber further comprises a collar proximate the first open end of the chamber, the collar comprising an inner and an outer surface.

The device may further comprises a first guide located within the collar. The first guide preferably comprises an annular outer surface which co-operates with the inner surface of the collar thereby forming a seal between the first guide and the inner surface of the collar. Preferably, the first guide further comprises an aperture for receiving the elongate member. The elongate member may be received by the first guide through the aperture such that a seal is formed between the external surface of the elongate member and the guide.

Preferably, the device further comprises a second guide located on the external surface of and proximate the first end of the elongate member. This second guide may be used to hold the elongate member in place during aspiration.

Since many biological reactions provide a quantitative result, it is preferable to regulate the volume of sample used in the reaction. Accordingly, in a further preferred embodiment, the device is adapted for use

in conjunction with a means for regulating the volume of the sample aspirated. The means for regulating the volume of the sample may be, for example, a manual or an electronic pipettor. It will be appreciated that currently available electronic pipettors may be used with the device of the present invention. Examples of such electronic pipettors include the UtlraMicroPump-II™ and the Nanoliter 2000™, both produced by World Precision Instruments.

In yet another preferred embodiment, the interior surface of the chamber and/or the elongate member is treated with a blocking agent in order to prevent the sample from adhering to the chamber or elongate member. The blocking agent may be selected from the group consisting of bovine serum albumin, fetal calf serum, human serum albumin, polyvinyl alcohol, polyvinyl pyrrolidone, a silicone compound and other suitable protein sources. Most preferably, the solution comprises a silicone compound sold under the trade name "Sigmacote" (Sigma).

In a further preferred embodiment, the elongate member is filled with oil in order to facilitate the uptake of the sample.

The present invention also provides a device which allows a series of separate reactions to be performed sequentially in the chamber.

Accordingly, in a second aspect, the present invention provides a device according to the first aspect, wherein the chamber comprises

a first reagent composition;

a first wax layer providing a seal over the first reagent composition;

a second reagent composition positioned adjacent the first wax layer and separated from the first reagent composition by the first wax layer; and

a second wax layer substantially covering the second reagent composition;

wherein the second wax layer has a melting temperature which is lower than the melting temperature of the first wax layer such that the first wax layer is solid at the melting temperature of the second wax layer.

In a preferred embodiment of the second aspect at least one of, more preferably each of, the first and second reagent compositions are in a liquid form at room temperature. However, it is also envisaged that the reagent composition(s) may be solid at room temperature. For example, the reagent composition(s) may be lyophilized.

In a third aspect, the present invention provides a device according to the first aspect, wherein the chamber comprises

a first reagent composition in admixture with a first wax carrier; and

a second reagent composition in admixture with a second wax carrier;

wherein the second reagent composition is at least partially coated, covered or layered upon the first reagent composition and wherein the second wax carrier has a melting temperature which is lower than the first wax carrier such that the first wax carrier is solid at the melting temperature of the second wax carrier.

In a fourth aspect the present invention provides a method for detecting a substance in a sample, the method comprising

(i) introducing a sample and a reagent composition into the chamber of a device according to the first aspect such that the sample and reagent composition form a reaction mix, wherein the reagent composition comprises at least one reagent which interacts with the substance to be detected such that the level of electromagnetic radiation emitted by the reaction mix is altered when compared to the level of electromagnetic radiation emitted by the reagent composition alone;

(ii) optionally exciting the reaction mix with electromagnetic radiation;

(iii) subjecting the chamber to conditions which allow the reaction to occur, and

(iv) detecting the electromagnetic radiation emitted by the reaction mix.

In a fifth aspect the present invention provides a method for amplifying at least one target nucleic acid molecule in a sample, the method comprising

(i) introducing a sample and a reagent composition into the chamber of a device according to the first aspect such that the sample and reagent composition form a reaction mix, wherein the reagent composition comprises reagents for amplification of the target sequence; and

(ii) subjecting the chamber to conditions which allow amplification of the target sequence.

In a sixth aspect the present invention provides a method for amplifying at least one target nucleic acid molecule in a sample, the method comprising

(i) introducing a sample and a reagent composition into the chamber of a device according to the first aspect such that the sample and reagent composition form a reaction mix,

wherein the reagent composition comprises reagents for amplification of the target sequence and at least one reagent which interacts with the amplified sequence such that the level of electromagnetic radiation emitted by the reaction mix is altered when compared to the level of electromagnetic radiation emitted by the reagent composition alone;

(ii) optionally exciting the reaction mix with electromagnetic radiation;

(iii) subjecting the chamber to conditions which allow amplification of the target sequence, and

(iv) detecting the electromagnetic radiation emitted by the reaction mix.

In a seventh aspect, the present invention provides a method for performing a series of reactions in a single vessel, the method comprising

(i) loading a sample into the chamber of a device according to the second aspect such that the sample is positioned adjacent the second wax layer;

(ii) heating the chamber to a temperature at which the second wax layer melts but the first wax layer remains solid;

(iii) allowing the sample to interact with the second reagent composition to form a reaction mix;

(iv) heating the chamber to a temperature at which the first wax layer melts; and

(v) allowing the reaction mix to interact with the first reagent composition.

In an eighth aspect the present invention provides a method for performing a series of reactions in a single vessel, the method comprising

(i) loading a sample into the chamber of a device according to the third aspect such that the sample is positioned adjacent the second reagent composition in admixture with the second wax carrier;

(ii) heating the chamber to a temperature at which the second wax carrier melts but the first wax carrier remains solid;

(iii) allowing the sample to interact with the second reagent composition to form a reaction mix;

(iv) heating the chamber to a temperature at which the first wax carrier melts; and

(v) allowing the reaction mix to interact with the first reagent composition.

In a preferred embodiment of the fourth to sixth aspects, the sample is introduced into the chamber through the elongate member.

In a preferred embodiment of the fourth and sixth aspects, electromagnetic radiation is transmitted from an external source through the chamber to the reaction mix. Furthermore, it is preferred that any alteration in the electromagnetic radiation of the reaction mix is detected through the chamber.

In a preferred embodiment of the sixth aspect the reagent composition comprises components which allow for the fluorescent based detection of an amplified nucleic acid molecule product by oligonucleotide hybridization.

In a preferred embodiment of the second and third aspects, the first wax layer has a melting temperature of about 70°C. Any wax which has a melting temperature of about 70°C may be suitable. Preferably, the wax is T Wax (EnerGene®) or F wax (20% paraflint C80N6 from Schumann Sasol, 80% T wax).

In a further preferred embodiment of the second and third aspects, the second wax layer has a melting temperature of about 55°C. Any wax which has a melting temperature of about 55°C may be suitable. Preferably, the wax is AmpliWax® (PE Applied Biosystems).

In yet a further preferred embodiment of the second and third aspects, an oil layer covers the second wax layer. The presence of the oil layer may perform one or more of a number functions, including maintaining a sample as a bolus adjacent the second wax layer, assisting transfer of the sample from the elongated member to the chamber, and reducing contamination.

In yet a further preferred embodiment of the second and third aspects, the first reagent composition comprises reagents required to amplify a target nucleic acid molecule. More preferably, the first reagent composition comprises components which allow for fluorescent based detection of an amplified nucleic acid product by oligonucleotide hybridization.

In yet a further preferred embodiment of the second and third aspects, the second reagent composition comprises components which result in cell lysis, protein digestion and/or modification, and/or DNA digestion and/or

modification. Preferably, the second reagent composition comprises an alkaline buffer in order to enhance cell lysis. More preferably, the second reagent composition comprises proteinase K or protease in a buffer compatible with proteinase K or protease activity.

In a preferred embodiment of the seventh and eighth aspects, step (iii) and/or step (v) include the step of centrifuging the elongate chamber. Preferably, the centrifugal force is between 5g and 200g, more preferably between 5g and 70g. However, as would be appreciated by those skilled in the art, the centrifugal force required will vary depending upon parameters such as the temperature of the chamber, the wax melting temperature, the viscosity of the compositions etc.

It will be appreciated by those skilled in the art that the chamber of a device of the second and third aspects may comprise additional reagent compositions and wax layers enabling more than two different reactions to occur in the same chamber.

In a preferred embodiment of the present invention, the sample comprises biological material. Preferably, the biological material comprises cells or viral particles.

In a further preferred embodiment of the present invention, the sample is selected from the group consisting of: red and white blood cells, embryonic cells, bacteria, sperm, pollen, cell cultures, pap smears, single cells, blood cell parasites, single cells infected with a virus, and other fluids containing DNA, RNA or protein.

It will be appreciated that the device of the first aspect of the present invention may also be used for injecting a sample into biological tissue or transferring a sample from one source to another.

Accordingly, in a ninth aspect, the present invention provides the use of a device of the first aspect for injecting a fluid sample into a cell. Preferably, the fluid sample comprises genetic material such as DNA or sperm.

In a preferred embodiment of the ninth aspect, the device is adapted for use in conjunction with a micromanipulator in order to control the introduction of the sample into the cell.

Throughout this specification, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but

not the exclusion of any other element, integer or step, or group of elements, integers or steps.

The invention will hereinafter be described by way of the following non-limiting Figures and Examples.

Brief Description of the Accompanying Drawings:

Figure 1 is a perspective view of a device in accordance with a preferred embodiment of the present invention in an assembled state.

Figure 2 is a cross-sectional view of a preferred device showing the elongate member in a first position.

Figure 3 is a cross-sectional view of a preferred device showing the elongate member in a second position.

Figure 4 is an exploded cross sectional view of a device of Figure 2.

Figure 5 is a cross-sectional view of a preferred device of the present invention showing (a) an assembled device; and (b) an elongate member and sealing means.

Figure 6 shows the results of a sex determination assay performed on human white blood cells by direct aspiration and single step real time amplification and detection using a device in accordance with a preferred embodiment of the present invention.

Detailed Description of Preferred Embodiments:

With reference to Figures 1 to 4, an aspiration device (1) constructed in accordance with a preferred embodiment of the present invention will now be described. The device comprises a chamber (2) having a first open end (3) and a second closed end (4), and an inner (5) and outer (6) surface. The chamber is cylindrical and has an outer diameter of up to 2.8 mm, an inner diameter of up to 2.5 mm and a length of between 20 and 40 mm. Preferably, the chamber can hold sample volumes of up to 20μl.

It is preferred that the chamber (2) is made of a material that is chemically inert, biocompatible and stable. Chemically inert means that the material is stable to deformation, discloroation, cracking, splitting etc. upon exposure to heat, autoclaving, extraction reagents, diluents or other chemical solutions. Biocompatible means that the material does not bind biological materials from a solution, affect the stability, functionality, or conformation of a biological material upon contact with that material, or in any way contaminate the biological solution with components that leach from the material in the biological solution. Stable means that the material retains all of the above characteristics for years at room temperature.

Preferred polymers include polypropylene, polyethylene terephthalate and polycarbonates, from which the entire device can be molded. Many grades of polypropylene are commercially available. A resin like Himont PD701 natural (Himont USA, Inc., Wilmington, Del.) is preferred as it exhibits sufficient inertness and can be autoclaved. Examples of suitable polycarbonates include, makrolon (Bayer), calibre (Dow Chemicals), lexan (GE) and acrifix 192 (Rohm Chemical Fabrik). The entire device may be injection molded under high injection pressures. Alternatively, the elongate chamber may be made of other materials such as glass.

It is preferred that the material allows transmission of electromagnetic radiation. Alternatively or in addition, the second closed end (4) of the chamber comprises a lens (7) for transmitting electromagnetic radiation.

The device further comprises an elongate member (8) having first (9) and second (10) open ends and an inner (11) and outer (12) surface. The elongate member is slidably movable from a first position (13) to a second position (14) in the elongate chamber (2). The first end (9) comprises a needle tip (15). Preferably, the needle tip has a diameter of between 1μm and

200μm. More preferably, the tip has a diameter of between 1μm to 50μm. Preferably, the needle tip is capable of piercing biological tissue, such as embryo tissue. Accordingly, it is preferred that at least the needle tip portion of the elongate member is made of glass. In one preferred embodiment, the entire elongate member (8) is made of glass.

A sealing means (16) provides a seal between the outer surface (12) of the elongate member and the inner surface (5) of the chamber (2). The sealing means comprises a sleeve (17) located on the outer surface (12) of the elongate member (8) proximate the second end (10). Teflon is preferably used as the material for the sleeve (17) although other materials such as rubber, plastic or glass may be used. The sleeve (17) co-operates with the inner surface (5) of the chamber (2) so as to provide a seal between the sleeve (17) and the inner surface (5) of the chamber. The sleeve may co-operate with the inner surface of the chamber along the entire length of the sleeve or along a substantial portion of the length of the sleeve. Figure 5 shows an alternative embodiment in which the sleeve (29) comprises a flared portion (30) which co-operates with the inner surface (31) of the chamber (32) to provide a seal.

In use, a fluid sample may be introduced into the chamber (2) via the elongate member (8) by movement of the elongate member (8) from a first position (13) to a second position (14). This movement may be achieved by sliding the chamber (2) away from the first end (9) of the elongate member (8). It will be appreciated that this movement of the chamber (2) relative to the elongate member (8) causes a decrease in pressure within the chamber (2) which draws the sample through the elongate member (8) into the chamber (2).

The chamber further comprises a raised collar (18) located on the chamber (2) proximate the first open end (3). The raised collar has an inner (19) and an outer (20) surface . The outer surface (20) may be held by the user in order to slide the chamber (2) away from or toward the first end (9) of the elongate member (8).

The device further comprises a first guide (21) located within the raised collar (18) . The first guide comprises an annular outer surface (22) which engages the inner surface (19) of the collar (18) thereby forming a seal between the annular outer surface (22) and the inner surface (19). The first guide further comprises an aperture (23) for receiving the elongate member

(8). The elongate member (8) is received by the first guide (21) through the aperture (23) such that the elongate member (8) is positioned co-axially with respect to the chamber (2). The external surface (12) of the elongate member (8) and the surface defining the aperture co-operate to form a seal. It is preferred that the first guide is made of silicon.

The device further comprises a second guide (24) located on the external surface (12) of and proximate the first end (9) of the elongate member (8). This second guide (24) may be used to hold the elongate member (8) in place whilst the chamber (2) is slidably moved away from or toward the first end (9) of the elongate member (8).

A preferred device (1) of the present invention will now be described generally in use.

The device of the present invention may be used to inject a fluid sample into a cell. It will be appreciated that sliding the chamber (2) toward the first end (9) of the elongate member (8) will increase the pressure within the chamber (2) and cause expulsion of any fluid in the chamber (2) out through the elongate member (8). In this manner, the device may be used to inject foreign genetic material, such as DNA or sperm, into a cell.

It is preferred, however, that the device of the present invention is used for aspiration of a sample into the chamber (2) and subsequent analysis of the sample. This analysis may be achieved by performing a desired reaction on the sample within the chamber. In the context of this embodiment, the elongate chamber may be loaded with a unit dose reagent composition necessary to perform a reaction. "Unit dose" refers to a reagent composition comprising all or nearly all of the reagents needed to accomplish a reaction except for the sample to be analysed. Preferably, the user need only add the sample in order to start the reaction. The reaction composition may be pre-loaded into the chamber (2) before the device is assembled. Alternatively, the reaction composition may be drawn into the chamber (2) through the elongate member (8). Preferably, the sample is drawn separately into the chamber (2) through the elongate member after the chamber has been loaded with the reagent composition. The reagent composition and sample then react within the chamber (2) to form a reaction mix.

The inner surface (5) of the chamber (2) and/or the inner surface (11) of the elongate member (8) may be treated with a blocking agent in order to facilitate the loading of a sample into the chamber (2). By "blocking agent"

we mean an agent which reduces or prevents the binding of biological materials in a solution to the inner surface (11) of the elongate member (8) or inner surface (5) elongate chamber (2). The inner surface (11) of the elongate member (8) or inner surface (5) of the chamber (2) may be contacted with the blocking agent in order to inactivate any binding sites prior to the aspiration of the sample. Alternatively or in addition, the blocking agent may be added directly to the sample prior to aspiration. The presence of the blocking agent in the sample may facilitate the progression of the sample along the elongate member during aspiration. The blocking agent may be a surfactant. Examples of suitable blocking agents include, but are not limited to, bovine serum albumin, fetal calf serum, human serum albumin, polyvinyl alcohol, polyvinyl pyrrolidone, and other suitable protein sources. Commercially available products suitable for use as blocking agents in the context of the present invention would be known by those skilled in the art. Suitable examples include, "Sigmacote" (Sigma) and "Vigro Retrieval".

The elongate member (8) may also be filled with oil in order to facilitate the passage of the sample through the elongate member into the chamber.

Although a device (1) according to the present invention may be used to perform a variety of reactions, in a preferred embodiment the device (1) is used for collecting a biological sample and analysing that sample for the presence of a target molecule. Preferably, the nature of the reagent composition is such that the presence of the target molecule in the biological substance may be detected by a change in the level of electromagnetic radiation emitted by the reaction mix.

In a further preferred embodiment, the target molecule is a nucleic acid sequence. Preferably, the reagent composition comprises reagents which allow amplification of the target nucleic acid molecule. Preferably, the reagent composition comprises at least one reagent which interacts with the amplified sequence to cause a change in the level of electromagnetic radiation emitted by the reaction mix.

An "amplification" reaction is a reaction in which multiple copies of an original nucleic acid sequence are generated, typically by repeating an enzymatic duplication process for a number of cycles. When additional copies can be made from each of the duplicate copies made in an earlier cycle, the amplification process is said to be exponential with respect to the

number of cycles. While exponential amplification is desirable to improve assay sensitivity, this heightened degree of sensitivity is also a drawback if the amplification products are not carefully contained, resulting in contamination.

Techniques for amplifying nucleic acid molecules are well known to those skilled in the art. Examples of these techniques are the polymerase chain reaction (PCR), disclosed in U.S. Patent Nos. 4,683,202 and 4,683,195 (Mullis); the ligase chain reaction (LCR) disclosed in EP-A-320 308 (Backman et al); and gap filling LCR (GLCR) or variations thereof, which are disclosed in WO 90/01069 (Segev), EP-A-439-182 (Backman, et al), GB 2,225,112A (Newton, et al) and WO 93/00447 (Birkenmeyer et al). Other amplification techniques include Q-Beta Replicase, as described in the literature; Strand Displacement Amplification (SDA) as described in EP-A-497 272 (Walker), EP-A-500 224 (Walker et al) and in Walker, et al., in Proc. Nat. Acad. Sci. U.S.A., 89:392(1992); Self-Sustained Sequence Replication (3SR) as described in Fahy, et al., in PCR Methods and Applications 1:25 (1991); and Nucleic Acid Sequence-Based Amplification (NASBA) as described in the literature.

Some amplification reactions, for example PCR and LCR, involve cycles of alternately high and low set temperatures, a process known as "thermal cycling". PCR or "Polymerase Chain Reaction" is an amplification reaction in which a polymerase enzyme, usually thermostable, generates multiple copies of the original sequence by extension of a primer using the original nucleic acid as a template. PCR is described in more detail in U.S. Pat. Nos. 4,683,202 and 4,683,195. LCR or "Ligase Chain Reaction" is a nucleic acid amplification reaction in which a ligase enzyme, usually thermostable, generates multiple copies of the original sequence by ligating two or more oligonucleotide probes while they are hybridized to the target. LCR, and its variation, Gap LCR, are described in more detail in EP-A-320-308 (Backman et al), EP-A-439-182 (Backman, et al) and WO 90/100447 (Birkenmeyer et al.) and elsewhere.

In addition, amplification techniques which include assays which involve fluorescent based detection are known to those skilled in the art. Examples of these include the Molecular Beacons assay (Tyagi and Kramer, 1996), TaqMan™ fluorescent energy transfer assay (Livak et al., 1995) and hybridization probes which result in Forster Resonance Energy Transfer (Deniz A.A. et al., Proc. Natl. Acad. Sci., USA 96:3670 (1999).

Once the reagent composition and sample have been loaded into the chamber (2), the elongate member (8) can be sealed, preferably by cutting the elongate member (8) at a point proximal to the chamber (2) followed by heat sealing. For example, the elongate member (8) may be simultaneously cut and sealed by the application of heat. Alternatively, the elongate member (8) may be removed from the chamber (2) and the open end (3) of the chamber (2) may be heat sealed or sealed by the application of a cap.

The sealed chamber may then be placed in a thermal cycler so that the target nucleic acid molecule in the sample undergoes amplification. At the same time, emitted electromagnetic radiation may be detected through the walls of the chamber or the lens in the base of the chamber.

The device and method of the present invention is suitable for use with a number of direct reaction detection technologies/chemistries such as Taqman (Perkin-Elmer), molecular beacons and the LightCycler™ fluorescent hybridization probe analysis (Roche Molecular Systems).

A preferred system for real time DNA amplification and detection is the LightCycler™ fluorescent hybridization probe analysis. This system involves the use of three essential components: two different oligonucleotides (labelled) and the amplification product. Oligonucleotide 1 carries a fluorescein label at its 3' end whereas oligonucleotide 2 carries another label, LC Red 640 or LC Red 705, at its 5' end. The sequences of the two oligonucleotides are selected such that they hybridize to the amplified DNA fragment in a head to tail arrangement. When the oligonucleotides hybridize in this orientation, the two fluorescence dyes are positioned in close proximity to each other. The first dye (fluorescein) is excited by the LightCycler's LED (Light Emitting Diode) filtered light source, and emits green fluorescent light at a slightly longer wavelength. When the two dyes are in close proximity, the emitted energy excites the LC Red 640 or LC Red 705 attached to the second hybridization probe that subsequently emits red fluorescent light at an even longer wavelength. This energy transfer, referred to as FRET (Forster Resonance Energy Transfer, or Fluorescence Resonance Energy Transfer) is highly dependent on the spacing between the two dye molecules. Only if the molecules are in close proximity (a distance between 1-5 nucleotides) the energy is transferred at high efficiency. Choosing the appropriate detection channel, the intensity of the light emitted by the LC Red 640 or LC Red 705 is filtered and measured by optics in the

thermocycler. The increasing amount of measured fluorescence proportional to the increasing amount of DNA generated during the ongoing PCR process. Since LC Red 640 and LC Red 705 only emits a signal when both oligonucleotides are hybridized, the fluorescence measurement is performed after the annealing step. Using hybridization probes can also be beneficial if samples containing very few template molecules are to be examined. DNA-quantification with hybridization probes is not only sensitive but also highly specific. It can be compared with agarose gel electrophoresis combined with Southern blot analysis but without all the time consuming steps which are required for the conventional analysis.

The 'TaqMan' fluorescence energy transfer assay uses a nucleic acid probe complementary to an internal segment of the target DNA. The probe is labelled with two fluorescent moieties with the property that the emission spectrum of one overlaps the excitation spectrum of the other; as a result the emission of the first fluorophore is largely quenched by the second. The probe is present during PCR and if PCR product is made, the probe becomes susceptible to degradation via a 5'-nuclease activity of Taq polymerase that is specific for DNA hybridized to template. Nucleolytic degradation of the probe allows the two fluorophores to separate in solution, which reduces the quenching and increases intensity of emitted light.

Probes used as molecular beacons are based on the principle of single-stranded nucleic acid molecules that possess a stem-and-loop structure. The loop portion of the molecule is a probe sequence that is complementary to a predetermined sequence in a target nucleic acid. The stem is formed by the annealing of two complementary arm sequences that are on either side of the probe sequence. The arm sequences are unrelated to the target sequence. A fluorescent moiety is attached to the end of one arm and a non-fluorescent quenching moiety is attached to the end of the other arm. The stem keeps these two moieties in close proximity to each other, causing the fluorescence of the fluorophore to be quenched by fluorescence resonance energy transfer. The nature of the fluorophore-quencher pair that we prefer to use is such that energy received by the fluorophore is transferred to the quencher and dissipated as heat, rather than being emitted as light. As a result, the fluorophore is unable to fluoresce. When the probe encounters a target molecule, it forms a hybrid that is no longer and more stable than the hybrid formed by the arm sequences. Since nucleic acid double helices are

relatively rigid, formation of a probe-target hybrid precludes the simultaneous existence of a hybrid formed by the arm sequences. Thus, the probe undergoes a spontaneous conformational change that forces the arm sequences apart and causes the fluorophore and quencher to move away from each other. Since the fluorophore is no longer in close proximity to the quencher, it fluoresces when illuminated by an appropriate light source. The probes are termed "molecular beacons" because they emit a fluorescent signal only when hybridized to target molecules.

A number of real time fluorescent detection thermocyclers are currently available with the chemistries being interchangeable with those discussed above as the final product is emitted fluorescence. Such thermocyclers include the Perkin Elmer Biosystems 7700, Corbett Research's Rotogene, and the Hoffman La Roche Light Cycler. It is envisaged that any of the above thermocyclers could be adapted to accommodate the device, and perform the method, of the present invention.

In one preferred embodiment, the device of the present invention may be used to perform a series of sequential reactions in a single chamber. In this embodiment, the elongate chamber (2) is preloaded with two or more reagent compositions, the reagent compositions being separated by layers of wax.

Figure 1 depicts a preloaded device suitable for performing sequential reactions. A first reagent composition (25) is covered by a first wax layer (26). A second reagent composition (27) is loaded on top of the first wax layer (26) and is covered by a second wax layer (28). The first wax layer (26) has a higher melting point temperature than the second wax layer (28). Oil (29) may be overlayed on the second wax layer (28).

It will be appreciated that a chamber (2) preloaded with reagent compositions as depicted in Figure 1 may be stored prior to use in a device (1) according to the present invention. The open end of the chamber (3) may be sealed with a cap prior to storage. The wax layers, when solidified in the chamber, preferably remain attached to the inner surface of the chamber during routine shipping and handling.

When used herein, "wax" refers to a wax-like organic substance, solid but much harder than greases at temperatures below about 40°C, which melts at somewhat higher temperatures to form a liquid and which has a lower density than water. Waxes may be composed of hydrocarbons, alcohols, fatty

acids and esters. They can be from plant, animal or mineral origin or may be synthetic. Examples of mineral waxes include Petroleum wax and Montan wax. Examples of synthetic waxes include Fischer-Tropsch waxes and Polyolefin waxes. Preferably, waxes contain principally esters of fatty acids and higher alcohols, free fatty acids and alcohols, and saturated hydrocarbons. Typical pure compounds which are useful waxes include eicosane, octacosane, cetyl palmitate, and pentaerythritol tetrabehenate. Wax-like substances include, for example, polyester gels. Non-silicone thixotropic gel-like substances may be used as wax-like substances. For example, a hydrocarbon gel-like material polybutene H-100, marketed by Amoco Chemicals Corporation, Chicago, III., and described in that company's bulletin as 12-H as butylene polymer composed predominantly of high molecular weight mono-olefins (85-98%), the balance being isoparaffins, when mixed with AEROSIL OX50, a fumed silica powder marketed by Degussa, Inc., Pigments Division, New York, N.Y., may be suitable. Another example of a useful hydrocarbon gel-like material is Poly bd® R-45HT, marketed by ARCO Chemical Company, New York, N.Y. and described in that company's general bulletin of April, 1976 as a hydroxyl terminated homopolymer of butadiene with the degree of polymerization being in the range of 50. Another example of a suitable gel-like material is a mixture of silicon fluid and very fine hydrophobic silicon dioxide powder.

Typical useful wax mixtures include paraffin, Paraplast (tradename of Sherwood Medical), Ultraflex (tradename of Petrolite Corporation), and BeSquare 175 (tradename of Petrolite Corporation). Particularly useful waxes for the present invention include; T wax™ (Energene, Regensburg, Germany), AmpliWax™ (PE Applied Biosystems, and Polyester Wax (Electron Microscopy Sciences). Waxes can be prepared by mixing pure or mixed waxes with one another or with greases or oils in any ratios which preserve the relative hardness and stickiness characteristic of a wax. Preferably, waxes used in the present invention are sterilized before use, using any method known effective for this purpose.

The amount of wax required for the present invention is that amount sufficient to cover the surface of the reagent composition. The amount can be determined by routine experimentation. The amount varies depending on several parameters, including, for example, the size of the reaction chamber.

For example, 0.015 to 0.025 g of wax in a 0.2 ml microtube, or 0.023 to 0.04 g of wax in a 0.5 ml microtube are useful.

"Oil" refers to a water-immiscible organic substance, liquid at temperatures below about 40°C, which has a lower density than water. "Mineral oil," also known as liquid petrolatum and paraffin oil, is a colourless, optically clear, mixture of high-molecular weight hydrocarbons with a density near 0.84 g/ml, widely available commercially and commonly used as a vapour barrier over PCR reactions. Melting Point Bath Oil by Sigma (silicon fluid) (catalogue no. M6884, density 0.96 g/ml or catalogue no. M9389, density 1.05 g/ml) may also be suitable.

A biological sample may be drawn into the chamber (2) through the elongate member (8) by movement of the chamber (2) relative to the elongate member (8) as described above. It will be understood that a sample introduced into the chamber (2) in this manner will be loaded adjacent the second wax layer (28). The chamber (2) may then be sealed and heated to a temperature at which the second wax layer (28) melts but the first wax layer (26) remains solid. This melting of the second wax layer (28) preferably brings the sample into fluid communication with the second reagent composition (27). The chamber (2) may be centrifuged at this stage in order to ensure admixing of the sample with the second reagent composition (27) to form a reaction mix. The centrifugal force may be between 5 to 200g, more preferably between 5 and 70g. The chamber (2) may then be subject to conditions which allow the desired reaction to occur.

The chamber is then heated to a temperature at which the first wax layer (26) melts. This melting of the first wax layer (26) preferably brings the reaction mix into fluid communication with the first reagent composition (25). The chamber (2) may be centrifuged at this stage as described above in order to ensure admixing of the reaction mix with the first reagent composition (25). The chamber (2) may then be subject to conditions which allow the desired reaction to occur.

It is also envisaged that the loaded chamber may be sealed and inverted prior to melting of the wax layers and mixing of the sample and reagents. For example, after the sample has been aspirated into the chamber, the open end of the chamber may be sealed before inverting the chamber and placing the inverted chamber in a thermal cycler. In this case, it is preferred that the collar is removed from the chamber. The same sequence of events as

described above will occur in this embodiment. The second and first reagent compositions will descend toward the sample in a temperature dependent manner, however, rather than the sample descending towards the reagent compositions.

As discussed above, in a preferred embodiment the device of the present invention is used for detecting a target nucleic acid molecule in a biological sample. Accordingly, it is preferred that the first reagent composition (25) comprises reagents which allow amplification of a target nucleic acid molecule.

As will be understood by those skilled in the art, the presence of proteins, particularly DNA degrading enzymes, in a biological sample can significantly hinder nucleic acid amplification reactions. Furthermore, it is important to liberate the nucleic acid from a cell, as well as to liberate the nucleic from binding proteins such as histones, in order to ensure that the nucleic acid is accessible to the polymerase in the amplification reaction composition.

Accordingly, it is preferred that the second reagent composition (27) comprises reagents which result in protein digestion and/or modification and which lead to the liberation of nucleic acid molecules from cellular components. It will be appreciated that interaction of the sample with a second reagent composition (27) such as this results in cellular lysis, protein digestion and release of nucleic acid molecules prior to performing an amplification reaction. Moreover, the exposure of the chamber to heat in order to melt the first wax layer (26) may inactivate any proteinase enzymes present in the second reagent composition prior to performing the amplification reaction. Accordingly, this embodiment allows the user to perform efficient and reliable amplification reactions on biological samples. Because the cellular digestion and amplification reactions occur sequentially in the same chamber, the risk of contamination is significantly reduced.

It will be appreciated that the chamber (2) may comprise more than two reagent compositions separated by wax layers. It is envisaged, for example, that a plurality of wax layers, each wax layer having a different melting point, may be used to separate a plurality of reagent compositions. This will allow more than two separate reactions to be performed sequentially within the chamber.

It will be appreciated that the device and methods of the present invention will be useful in a range of applications such as forensic analysis, diagnostic genetic tests, examining gene expression in single cells and sex determining tests. In particular, it is envisaged that the reaction chamber and methods of the present invention will be useful for reactions performed on small samples, such as single cells, embryonic cells, sperm, pollen, blood cell parasites, single cells infected with a virus, and DNA containing fluids.

Example 1 : Preparation of aspiration device.

A PCR reaction mix (17µl) was added to a chamber with a diameter of approximately 1.6mm. The 17µl PCR reaction mix contained DNA polymerase, reaction buffer including nucleotides and $MgCl_2$ (TaqMan Universal PCR Master Mix - PE Applied Biosystems), and two sets of oligonucleotide primers, one set specific for the target region (SRY HMG Box) of the Human Y chromosome and the other set of primers specific for the autosomal target region G6PD on the Human X chromosome. The PCR primers were each present at a final concentration of 0.3µM. The reaction mix also contained PCR product detection probes each at a concentration of 0.2µM. One probe detects products amplified from the Human Y chromosome, whereas the other probe detects products amplified from the Human X chromosome.

F wax (20% paraflint C80N6 from Schumann Sasol, 80% T wax) with a melting temperature of about 76°C was heated to 90°C and 3.3µl aspirated using a Drummond Capillary Positive Displacement Pipette, allowed to solidify, and expelled into the capillary device. The device was then loaded onto a Rotogene (Corbett Research), centrifuged for 30 seconds at 78°C, removed from the machine and cooled to 4°C, thus providing a solid wax layer covering the PCR reaction mix.

2.5µl of proteinase K mix (2x Gold PCR buffer (Perkin Elmer), 0.4mg/ml proteinase K (Boehringer Mannheim), 18uM SDS and the balance water) was then layered on the top of the solidified F wax. A wax (AmpliWax Gem 50, PE Applied Biosystems) with a melting temperature of about 55°C was then added to the device in the same manner as the F wax, however, the A wax was melted at 75°C and centrifuged for 30 seconds in the Rotogene at 60°C.

Finally, 15 µl of mineral oil (Sigma Chemicals) was loaded on top of the A wax.

This chamber was then assembled with a glass elongate member comprising a needle tip to produce a device as depicted in Figure 1.

Example 2: Detection of a target nucleic acid in Human White Blood Cells.

Human white blood cells (WBC) from either male or female Human were collected in cell preparation tubes with sodium citrate(CPT) (Becton Dickinson, Cat #362761) for separating WBC from red blood cells, and washed 2 times in phosphate buffered saline (0.01M phosphate buffer, 0.0027M KCl, 0.137M NaCl). The final WBC pellet was resuspended in "Vigro Retrieval" (A.B. Technology). The "Vigro Retrieval" functions to prevent the WBC adhering to the walls of the tube. The WBC were then placed in a petri dish, and overlayed with mineral oil for pickup.

The required number of WBC were in 2.5 µl of "Vigro Retrieval" preparation visually counted and aspirated using a device which had been prepared as described in Example 1. For controls, similar volumes of male or female Human DNA or "Vigro Retrieval" solution only (no DNA) were loaded in to separate devices.

The WBC were aspirated into the elongate chamber on top of the second wax layer underneath the oil and the glass elongate member was then removed. After capping the device was placed in a Rotogene thermocycler. To allow the first protein digestion reaction to occur the device was heated to 60°C for 10 minutes. In this step the A wax melted and the F wax remained solid. Following this reaction, the chamber was then heated to72°C for 10 mins in order to inactivate the proteinase K. The chamber was then exposed to the following PCR conditions: 1 x 94°C for 10 min (melting of the F wax layer occurs); 50 x 93°C for 15s, 60°C for 40s, 60°C for 10s.

The final results of the PCR are shown in Figure 6. The x-axis clearly indicates the type of sample- either isolated DNA or WBC, the source-either male or female, and the number of genomes or WBC added to the reaction chamber.

All control samples and 11 out of the 12 samples analysed gave the expected result for sex and were positive for the X internal control with the exception of the no DNA controls (samples 3 & 16) which was correct for no

sex or autosomal signal, and sample number 6 which failed to provide a sex or autosomal signal.

Notably, for each of the male WBC and DNA samples analysed, a signal was detected with no false positive signals detected in any of the female derived samples. Furthermore, as can be seen in samples 10 to 15, the method was of sufficient sensitivity to detect a single copy of a target sequence, namely a single Y chromosome sequence present in a single WBC.

Each of the patents, patent applications and literature documents specifically cited above is incorporated herein in its entirety by reference.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Claims:

1. A device comprising a chamber having a first open end and a second closed end and an inner and an outer surface, an elongate member having first and second open ends and having an inner and an outer surface, and sealing means providing a seal between the elongate member and the chamber, the second end of the elongate member being slidably movable from a first position within the chamber to a second position within the chamber, the movement of the second end of the elongate member from the first position to the second position causing a change in the pressure within the chamber.

2. A device according to claim 1, wherein the chamber is elongate.

3. A device according to claim 1 or claim 2, wherein the sealing means provides a seal between the outer surface of the elongate member and the inner surface of the chamber.

4. A device according to any one of claims 1 to 3, wherein the chamber allows transmission of electromagnetic radiation.

5. A device according to any one of claims 1 to 3, wherein the chamber is cylindrical.

6. A device according to claim 5, wherein the cylindrical chamber has an outer diameter of up to 2.8 mm.

7. A device according to claim 5 or claim 6, wherein the cylindrical chamber has an inner diameter of up to 2.5 mm.

8. A device according to any one of claims 5 to 7, wherein the cylindrical chamber has a length of between 20 to 40 mm.

9. A device according to any one of claims 1 to 8, wherein the first end of the elongate member comprises a formed tip.

10. A device according to claim 9, wherein the formed tip has a diameter of between 1μm and 200μm.

11. A device according to any one of claims 1 to 10, wherein the chamber and elongate member are formed of the same material.

12. A device according to claim 11, wherein the material is glass or plastic.

13. A device according to any one of claims 1 to 10, wherein the chamber is made of a plastic and the elongate member is made of glass or vice versa.

14. A device according to claim 12 or claim 13, wherein the plastic is selected from the group consisting of polyethylene terephthalate, polypropylene and polycarbonate.

15. A device according to any one of claims 1 to 14, wherein the sealing means comprises a sleeve located on the outer surface of the elongate member proximate the second end of the elongate member.

16. A device according to any one of claims 1 to 15, wherein the chamber further comprises a raised collar proximate the open end of the chamber.

17. A device according to claim 16 further comprising a guide located within the raised collar, the guide containing an aperture for receiving the elongate member.

18. A device according to claim 17, wherein the elongate member is received by the aperture such that a seal is formed between the elongate member and the guide.

19. A device according to any one of claims 1 to 18, wherein the inner surface of the chamber and/or the inner surface of the elongate member is at least partially coated with a blocking agent.

20. A device according to claim 19, wherein the blocking agent comprises at least one component selected from the group consisting of bovine serum albumin, fetal calf serum, human serum albumin, polyvinyl alcohol, polyvinyl pyrrolidone and a silicone compound.

21. A device according to any one of claims 1 to 20, wherein the second end of the chamber comprises a lens for transmitting electromagnetic radiation.

22. A device according to any one of claims 1 to 21, wherein the chamber contains

a first reagent composition;

a first wax layer providing a seal over the first reagent composition;

a second reagent composition positioned adjacent the first wax layer and separated from the first reagent composition by the first wax layer; and

a second wax layer substantially covering the second reagent composition;

wherein the second wax layer has a melting temperature which is lower than the melting temperature of the first wax layer such that the first wax layer is solid at the melting temperature of the second wax layer.

23. A device according to claim 22, wherein the first wax layer has a melting temperature of about 70°C.

24. A device according to claim 22 or claim 23, wherein the second wax layer has a melting temperature of about 55°C.

25. A device according to any one of claims 22 to 24, wherein the first reagent composition comprises reagents required for amplification of a target nucleic acid molecule.

26. A device according to any one of claims 22 to 25, wherein the second reagent composition comprises reagents which result in cell lysis, protein digestion and/or modification, or DNA digestion and/or modification.

27. A device according to claim 26, wherein the second reagent composition comprises an alkaline buffer in order to enhance cell lysis.

28. A device according to any one of claims 22 to 27, wherein the chamber further contains a third reagent composition positioned adjacent the second wax layer.

29. A device according to claim 28, wherein the chamber further contains a third wax layer positioned adjacent the third reagent composition.

30. A device according to any one of claims 1 to 21, wherein the chamber contains

a first reagent composition in admixture with a first wax carrier; and

a second reagent composition in admixture with a second wax carrier;

wherein the second reagent composition is at least partially coated, covered or layered upon the first reagent composition and wherein the second wax carrier has a melting temperature which is lower than the first wax carrier such that the first wax carrier is solid at the melting temperature of the second wax carrier.

31. A device according to claim 30, wherein the first wax carrier has a melting temperature of about 70°C.

32. A device according to claim 30 or claim 31, wherein the second wax layer has a melting temperature of about 55°C.

33. A device according to any one of claims 30 to 32, wherein the first reagent composition comprises reagents required for amplification of a target nucleic acid molecule.

34. A device according to any one of claims 30 to 33, wherein the second reagent composition comprises reagents which result in cell lysis, protein digestion and/or modification, or DNA digestion and/or modification.

35. A device according to claim 34, wherein the second reagent composition comprises an alkaline buffer in order to enhance cell lysis.

36. A device according to any one of claims 30 to 35, wherein the chamber further contains a third reagent composition in admixture with a third wax carrier, wherein the third reagent composition is at least partially coated, covered or layered upon the second reagent composition.

37. A method for detecting a substance in a sample, the method comprising

(i) introducing a sample and a reagent composition into the chamber of a device according to any one of claims 1 to 21 such that the sample and reagent composition form a reaction mix, wherein the reagent composition comprises at least one reagent which interacts with the substance to be detected such that the level of electromagnetic radiation emitted by the reaction mix is altered when compared to the level of electromagnetic radiation emitted by the reagent composition alone;

(ii) optionally exciting the reaction mix with electromagnetic radiation;

(iii) subjecting the reaction chamber to conditions which allow the reaction to occur, and

(iv) detecting the electromagnetic radiation emitted by the reaction mix.

38. A method for amplifying at least one target nucleic acid molecule in a sample, the method comprising

(i) introducing a sample and a reagent composition into the chamber of a device according to any one of claims 1 to 21 such that the sample and reagent composition form a reaction mix, wherein the reagent composition comprises reagents for amplification of the target sequence; and

(ii) subjecting the chamber to conditions which allow amplification of the target sequence.

39. A method for amplifying at least one target nucleic acid molecule in a sample, the method comprising

(i) introducing a sample and a reagent composition into the chamber of a device according to any one of claims 1 to 21 such that the sample and reagent composition form a reaction mix,

wherein the reagent composition comprises reagents for amplification of the target sequence and at least one reagent which interacts with the amplified sequence such that the level of electromagnetic radiation emitted by the reaction mix is altered when compared to the level of electromagnetic radiation emitted by the reagent composition alone;

(ii) optionally exciting the reaction mix with electromagnetic radiation;

(iii) subjecting the chamber to conditions which allow amplification of the target sequence, and

(iv) detecting the electromagnetic radiation emitted by the reaction mix.

40. A method according to claim 37 or claim 39, wherein electromagnetic radiation is transmitted from an external source through the chamber to the reaction mix.

41. A method according to claim 37 or claim 39, wherein the alteration in the electromagnetic radiation of the reaction mix is detected from the chamber.

42. A method according to any one of claims 37 to 41, wherein the reagent composition is introduced into the chamber through the elongate member.

43. A method according to any one of claims 37 to 42, wherein the sample is introduced into the chamber through the elongate member.

44. A method for performing a series of reactions in a single vessel, the method comprising

(i) loading a sample into the chamber of a device according to any one of claims 22 to 27 such that the sample is positioned adjacent the second wax layer;

(ii) heating the chamber to a temperature at which the second wax layer melts but the first wax layer remains solid;

(iii) allowing the sample to interact with the second reagent composition to form a reaction mix;

(iv) heating the chamber to a temperature at which the first wax layer melts; and

(v) allowing the reaction mix to interact with the first reagent composition.

45. A method for performing a series of reactions in a single vessel, the method comprising

(i) loading a sample into the chamber of a device according to any one of claims 30 to 35 such that the sample is positioned adjacent the second reagent composition;

(ii) heating the chamber to a temperature at which the second wax carrier melts but the first wax carrier remains solid;

(iii) allowing the sample to interact with the second reagent composition to form a reaction mix;

(iv) heating the chamber to a temperature at which the first wax carrier melts; and

(v) allowing the reaction mix to interact with the first reagent composition.

46. A method according to claim 44 or claim 45, wherein step (iii) and/or step (v) are achieved by centrifuging the chamber.

47. A method according to any one of claims 37 to 46, wherein the sample comprises biological material.

48. A method according to claim 47 wherein the biological material comprises cells or viral particles.

49. A method according to any one of claims 37 to 48, wherein the second reagent composition comprises reagents for protein digestion and/or cell lysis.

50. A method according to any one of claims 37 to 49, wherein the first reagent composition comprises reagents for amplification of a target nucleic acid molecule.

51. A method according to any one of claims 37 to 50, wherein the sample is selected from the group consisting of: red and white blood cells, embryonic cells, bacteria, sperm, pollen, cell cultures, pap smears, single cells, blood cell parasites, single cells infected with a virus, and fluids containing DNA, RNA or protein.

52. The use of a device according to any one of claims 1 to 21 for injecting a fluid sample into a cell.

53. The use as claimed in claim 52, wherein the fluid sample comprises genetic material.

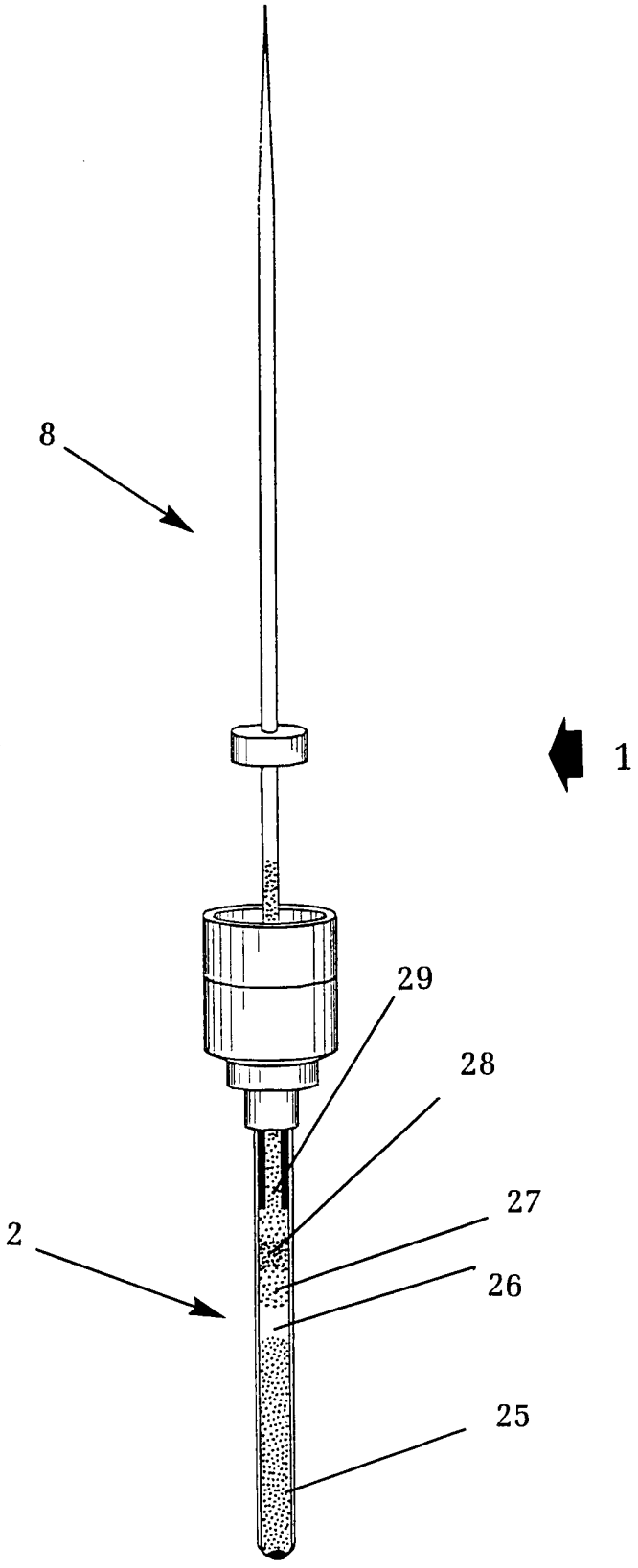


Figure 1

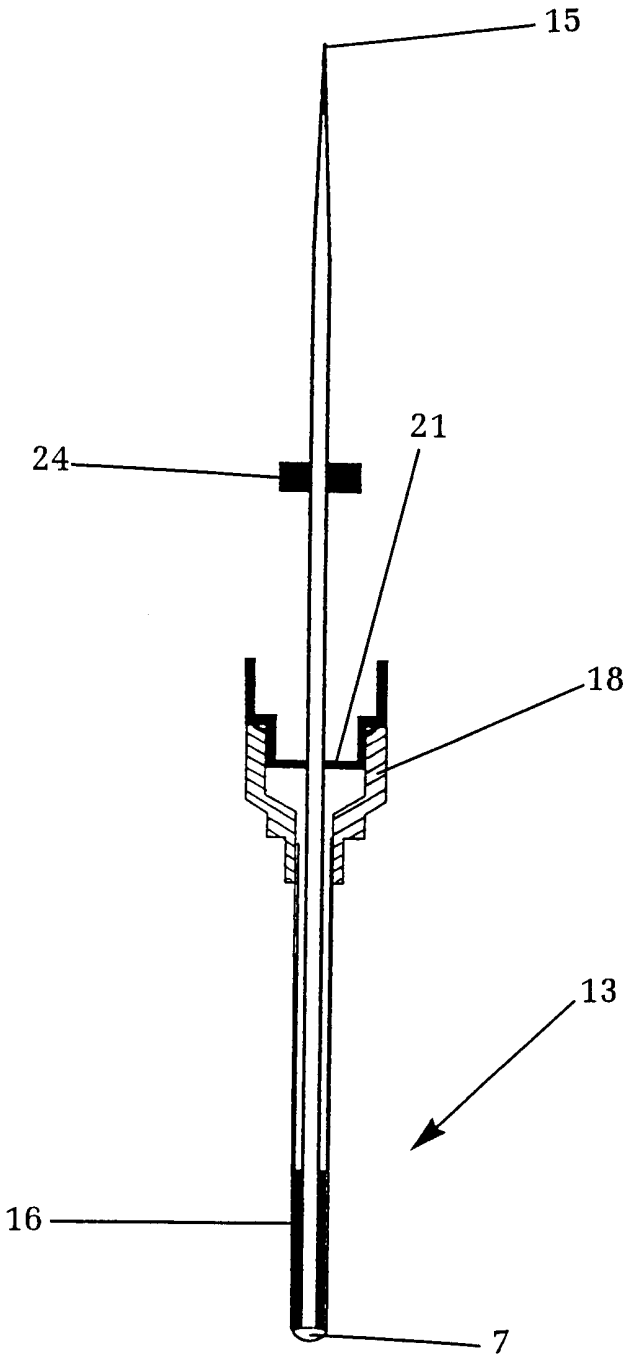


Figure 2

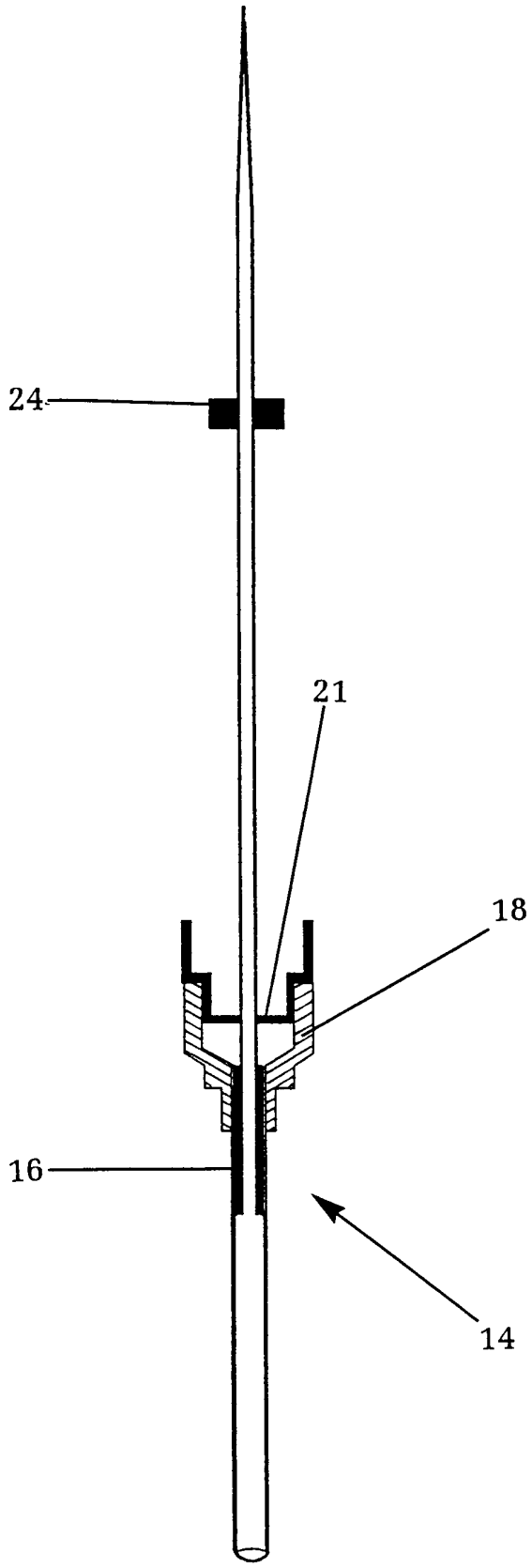


Figure 3

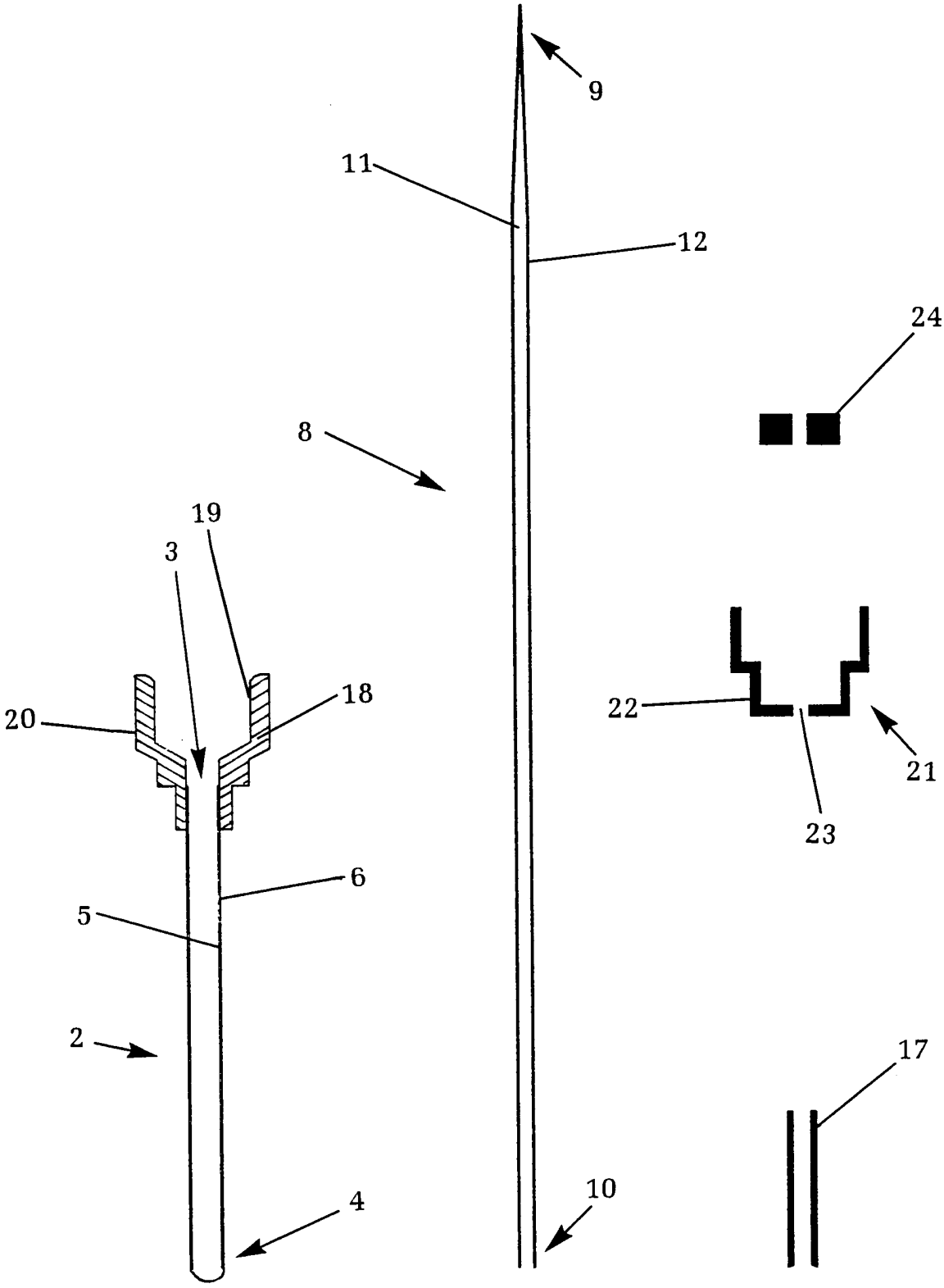


Figure 4

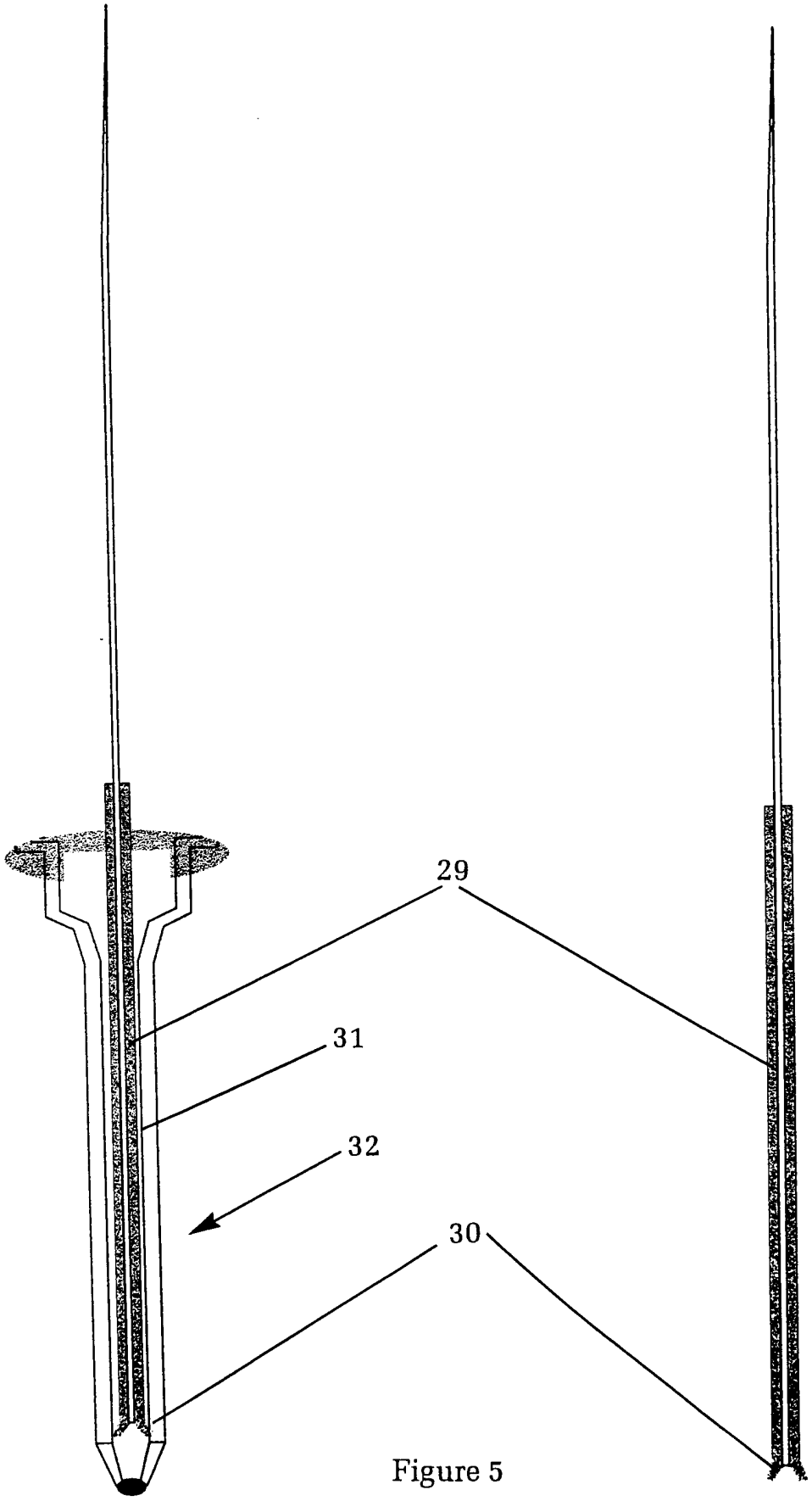


Figure 5

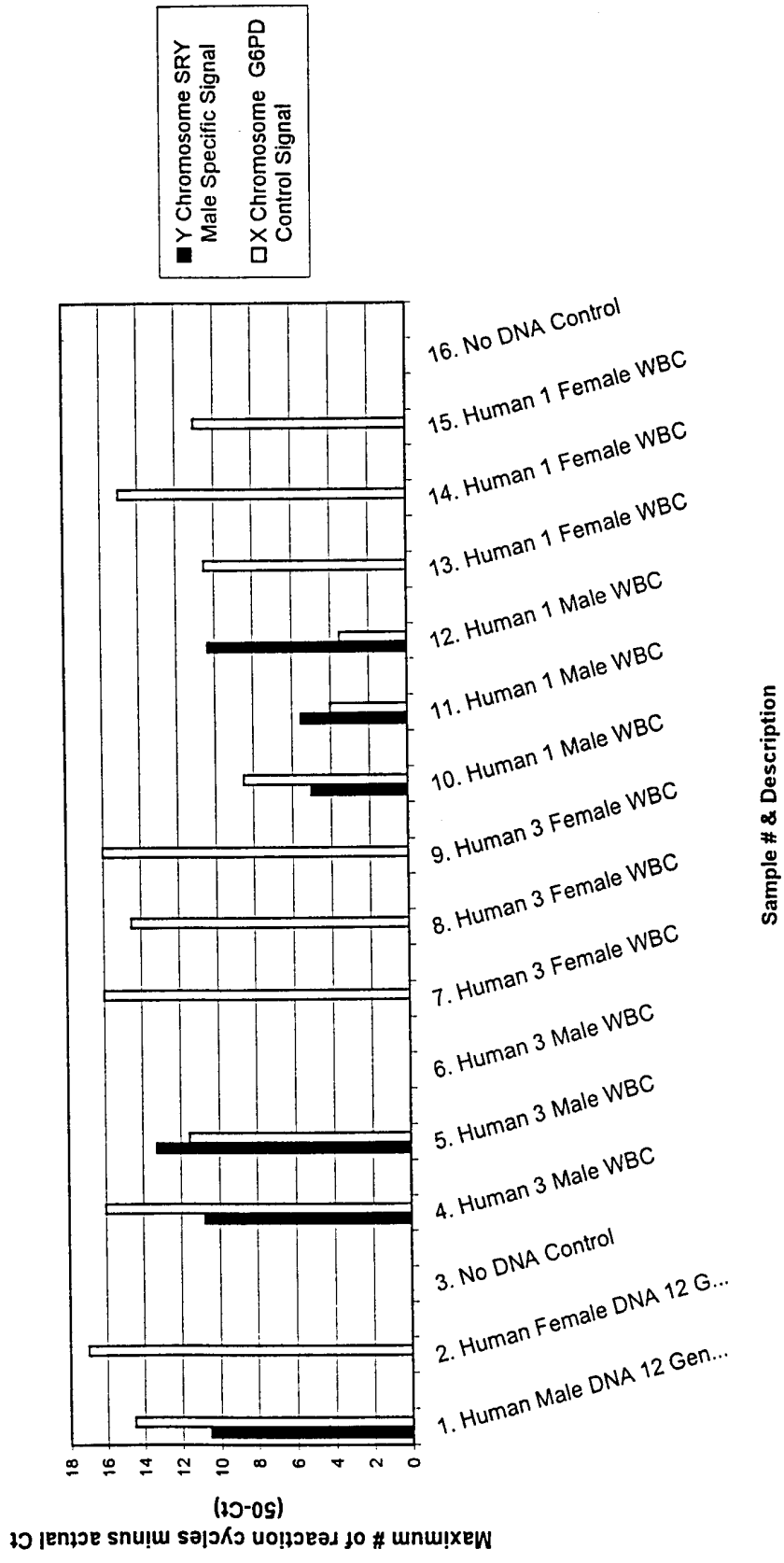

Y Chromosome SRY Male Specific Signal
X Chromosome G6PD Control Signal
16. No DNA Control
15. Human 1 Female WBC
14. Human 1 Female WBC
13. Human 1 Female WBC
12. Human 1 Male WBC
11. Human 1 Male WBC
10. Human 1 Male WBC
9. Human 3 Female WBC
8. Human 3 Female WBC
7. Human 3 Female WBC
6. Human 3 Male WBC
5. Human 3 Male WBC
4. Human 3 Male WBC
3. No DNA Control
2. Human Female DNA 12 G...
1. Human Male DNA 12 Gen...
Sample # & Description
18
16
14
12
10
8
6
4
2
0
(50-Ct)
Maximum # of reaction cycles minus actual Ct

Figure 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/00931

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: G01N 1/14 C12Q 1/68

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: G01N 1/-

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CA 1 113 275 A (MULL) 1 December 1981 figures 1 and 2, page 5 line 29 to page 6 line 15, page 9	1 - 9, 11 - 13, 16
P, A	US 5 968 729 A (KOSAK & KOSAK) 19 October 1999 whole document	
A	US 4 804 050 a (KERFOOT) 14 February 1989 whole document	

☐ Further documents are listed in the continuation of Box C ☒ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search	Date of mailing of the international search report
9 November 2000	10 NOV 2000

Name and mailing address of the ISA/AU	Authorised officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	10 NOV 2000 **Ross Burdon** Telephone No : (02) 6283

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU00/00931

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
CA	1 113 275		- none -				
US	5 968 729	AU	36191/93	CA	2 130 108	EP	626 017
		WO	93-16200				
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END OF ANNEX