The present invention provides a method and device for detection of harmful biological agents in an air sample.
DEVICE FOR DETECTION OF BIOLOGICAL AGENTS

FIELD OF THE INVENTION

The present invention provides a method and device for detection of harmful biological agents in an air sample.

BACKGROUND OF THE INVENTION

Nowadays the threat that biological warfare agents will be used on both military forces and civilian populations is more likely than it was at any other point in history. A number of countries have continued offensive biological weapons research and terrorist organizations have become possible users of biological warfare agents. Biological weapons may kill or harm individuals as well as hundreds of thousands of people and destruct entire cities or places. Biological agents are relatively easy and inexpensive to obtain or produce and in addition to physical damage can cause widespread fear and panic.

One of the dangers of biological agents lies in the fact that they are usually invisible, odorless, tasteless, and can be spread silently. Biological warfare agents include both living microorganisms (such as e.g. bacteria, viruses and fungi) and toxins produced by organisms, plants or animals and may for example be distributed by air, put into food or water or used in explosives.

Facing this threat, the destruction and sensing of biological weapons has become an important area of focus.

One of the most important and pressing problems involving spread of biological warfare agents is that of early detection and warning.

Currently available detection methods are only partially automated and analysis of samples is tedious and time-consuming. Therefore warning may come too late for those affected and spreading to other areas cannot be prevented in time.
Furthermore, current detection methods require an expensive laboratory infrastructure, specialized laboratory personnel and high safety measures in order to avoid exposure to highly contagious agents. Current methods often also lack the desired sensitivity thus leading to false alarms.

Therefore there is an ongoing need for reliable methods and devices which allow for an early, specific and highly sensitive detection of harmful biological agents.

OBJECTIVE AND SUMMARY OF THE INVENTION

It is an objective of the present invention to provide a method and device which allow for easy and early detection of airborne biological agents.

These and other objectives as they will become apparent from the ensuing description and claims are attained by the subject matter of the independent claims. Some of the preferred embodiments are defined by the dependent claims.

The method and device according to the invention provide the advantage that air samples can be collected and analysed on site, e.g. in an outdoor environment, since the device is portable. Furthermore, the method and device allow for continuous collection and analysis of air samples over long periods of time and thus for ongoing monitoring and surveillance of an area. This permits acquisition of multiple field data, such that confined areas of contamination can be quickly localized and evacuated or avoided if serious pathogens prevent access to such areas. The method and device according to the invention further provide the advantage that harmful biological agents can be quickly detected without unnecessarily exposing soldiers, laboratory workers or civilians to said agents.

In a first aspect the invention relates to a device for detecting a biological agent in an air sample, the device comprising:
(a) a particle concentration unit that collects particles from the air sample into a liquid;
(b) a liquid collection chamber comprising magnetic beads, wherein the magnetic beads are capable of binding polynucleotides;
(c) an amplification unit suitable for amplifying polynucleotides bound to the magnetic beads; and
(d) a detection system for detecting amplified polynucleotides.

In a further aspect the invention provides a method for detecting biological agents in an air sample comprising at least the steps of:

(a) providing an air sample;
(b) collecting particles from the air sample into a liquid;
(c) releasing polynucleotides from the particles into the liquid;
(d) binding the released polynucleotides to magnetic beads capable of binding said polynucleotides;
(e) subjecting the polynucleotides captured on the magnetic beads to polymerase chain reaction; and
(f) detecting and/or quantifying the amplified polynucleotides.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1-6 show exemplary and particularly preferred embodiments of the device of the invention. The electromagnet shown in figures 1 to 6 may also be replaced by a permanent magnet.

Fig. 1: Side view of preferred embodiment of device according to the invention.

Fig. 2: Top view of the device shown in Fig. 1 further showing amplification unit comprising rotatable disc.
Fig. 3: Particularly preferred embodiment of the device according to claim 4(c); inlet (1) illustrates lysis of biological agent; inlet (2) illustrates oligonucleotide primer extension on bead-surface and inlet (3) illustrates detection of amplified polynucleotides.

Fig. 4: Device shown in fig. 3, wherein the particle concentration unit, liquid collection chamber and amplification unit are shown in side view.

Fig. 5 and 6: Particularly preferred embodiment of the device according to claim 4(b). Illustrated is the amplification unit.

DETAILED DESCRIPTION OF THE INVENTION

Before the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary.

It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

In the following some definitions are introduced.

As used in this specification and in the intended claims, the singular forms of "a" and "an" also include the respective plurals unless the context clearly dictates otherwise. It is to be understood that the term "comprise", and variations such as "comprises" and "comprising" is not limiting.
For the purpose of the present invention the term "consisting of" is considered to be a preferred embodiment of the term "comprising". If hereinafter a group is defined to comprise at least a certain number of embodiments, this is meant to also encompass a group which preferably consists of these embodiments only.

If an oligonucleotide primer or any other polynucleotide described herein is said to be "specific" for a target polynucleotide or any other polynucleotide sequence or to "specifically" bind to or to "specifically" hybridize to a target polynucleotide or any other polynucleotide sequence, this refers to preferential binding, duplexing, or hybridizing of said primer or any other polynucleotide molecule to a particular polynucleotide sequence under stringent conditions. The term "stringent conditions" refers to conditions under which a probe or a primer will hybridize preferentially to its target sequence, and to a lesser extent to, or not at all to, other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances.

Stringent conditions may for example be selected to be about 5°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. For example, stringent conditions may be those in which the salt concentration is at least about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide.

The present invention in a first aspect relates to a device for detecting a biological agent in an air sample, the device comprising:

(a) a particle concentration unit that collects particles from the air sample into a liquid;
(b) a liquid collection chamber comprising magnetic beads, wherein the magnetic beads are capable of binding polynucleotides;
(c) an amplification unit suitable for amplifying polynucleotides bound to the magnetic beads; and
(d) a detection system for detecting amplified polynucleotides.

It is preferred that the device is suitable for installation on military equipment such as e.g. military ground vehicles (e.g. tanks), airplanes, military drones or missiles. Therefore, in one preferred embodiment the device according to the invention is portable. In a preferred embodiment the device is suitable for detection aboard an airborne drone.

The term "biological agent" as used herein refers to any pathogen, preferably selected from the group consisting of a virus, a spore, a fungus, a bacterium and a parasite, capable of causing disease, injury, or death in humans and/or animals. Examples of "biological agents" include e.g. Bacillus anthracis, Yersinia pestis, Vibrio cholerae, Coxiella burnetii, Francisella tularensis, Clostridium botulinum, Variola major, Lassa virus, Ebola virus and Marburg virus.

The term "air-sample" refers to a volume of air. In some embodiments said air sample may be pre-filtered e.g. using a coarse filter to prevent larger particles from entering the device. Larger particles may e.g. be particles larger than 0.5 mm. The air sample preferably enters the device via an air intake device which may for example be an opening such as a tube or a slit. In some embodiments, the air intake device may also comprise a pump which transports air from outside of the device into the device and, in particular into the particle concentration unit and most preferably into said liquid.

In one preferred embodiment the particle concentration unit comprises or consists of an electrostatic collection precipitator.
Preferably said electrostatic collection precipitator is a wet electrostatic precipitator. One example of a suitable electrostatic precipitator is e.g. disclosed in US 2004/0083790 which is hereby incorporated by reference. In a preferred embodiment the particle concentration unit comprises an air intake device.

The particles collected from the air sample into the liquid in (a) may be any particles comprised in the air sample, such as e.g. dust, microorganisms, or any other small particles that may be carried by the air. In a preferred embodiment particles collected from the air sample into the liquid in (a) are airborne biological agents. As used herein the term "liquid" refers to a water-based solution. In one preferred embodiment the liquid is a buffered solution or a nutrient solution. If the liquid is buffered, then it is preferred that it has a pH of between 6 and 8 and most preferably a pH of between 7 and 7.6.

In one particularly preferred embodiment, the liquid comprises

(i) a metal ion chelator such as EDTA or EGTA;
(ii) a buffering agent, such as Tris-Cl; and
(iii) water or phosphate buffered saline (PBS).

The device is preferably configured such that the liquid with the particles collected in said particle concentration unit enters said liquid collection chamber. In a preferred embodiment the particles collected in the particle concentration unit are lysed. In some embodiments such lysis may e.g. occur in said particle concentration unit, in said liquid collection chamber or in both. In some preferred embodiments lysis may also occur in the amplification unit.

If lysis takes place in the particle concentration unit, the liquid collection chamber or in both, preferably the liquid comprises a detergent.

In some embodiments the detergent may only be added once the liquid has entered the liquid collection chamber.
The detergent may be any ionic and/or anionic detergent known to the skilled person. In a particular preferred embodiment the detergent is selected from the group consisting of N-Lauroylsarcosin, sodium dodecyl sulphate (SDS) or any other alkali metal alkylsulphate salt, Triton X-100, CHAPS, TWEEN-20, TWEEN-80 or a combination of such detergents. In some embodiments the detergent may be used in concentrations of 0.2 to 30% (w/v), preferably in concentrations of 0.5 to 15% (w/v), more preferably in concentrations of 1 to 10% (w/v). In a particular preferred embodiment the detergent may be used in concentrations of 1.0 to 5% (w/v), even more preferred in concentrations of 0.5 to 5% (w/v).

The detergent may be comprised in any suitable buffer known to the skilled person including for example Tris buffer or phosphate buffer.

In some preferred embodiments the liquid may also comprise one or more lytic enzymes suitable for lysis of biological agents such as e.g. lysozyme, proteases, glucanase, glucosaminidase, muramidase or lysoamidase. The liquid may comprise said one or more lytic enzymes instead of or in addition to said detergent. In a preferred embodiment the liquid comprises lytic enzymes in addition to said detergent.

In some further preferred embodiments the liquid may also be temporarily heated, preferably to 60°-98°C in order to aid the lysis of collected biological agent(s). Optionally, the device of the invention also comprises a sonicator which sonicates the liquid with ultra-sound to further aid lysis of collected biological agent(s). In addition or alternatively, the rapid stirring of magnetic beads using alternating external magnetic fields can be used to break up the collected biological agent(s). The "liquid collection chamber" is preferably a container which accepts liquid from the particle concentration unit and which comprises magnetic beads that are capable of binding polynucleotides. These polynucleotides are preferably DNA or RNA polynucleotide that are released from the biological agents upon lysis.
Magnetic beads capable of binding polynucleotides as described herein preferably comprise one or more of the following features:

(i) the beads are positively charged; for example, the beads may be coated with a resin that is positively charged;
(ii) the beads comprise oligonucleotide primers on their surface capable of specifically hybridizing to target polynucleotides derived from a biological agent that has preferably been collected in the particle concentration unit; and/or
(iii) the beads comprise basic functional groups that have been covalently linked to the bead surface. Such basic functional groups may be e.g. \(-\text{NH}_3^+\) or \(-\text{NR}_3^+\); wherein \(R\) can e.g. be a Cl-C3-alkyl.

In a preferred embodiment the oligonucleotide primers in (ii) are covalently bound to the surface of the beads.

In a particularly preferred embodiment the magnetic beads consist of polymers into which magnetic nanoparticles (e.g. magnetite) are embedded. Examples of polymers that may be used are polyethylene glycol, polyacrylamide, polymethylmethacrylate, polyvinyl alcohol, styrene, N-isopropylacrylamide and others.

Magnetic beads as described herein are preferably spherical, but may have other shapes as well. For example, the beads may be oval, elliptical, disk-shaped, ring-shaped or square.

In one preferred embodiment, the liquid collection chamber further comprises magnetic beads which carry antibodies that are covalently linked to the surface of the beads. Said antibodies preferably bind proteins on the surface of specific biological agents.
Such preferred mixture of magnetic beads capable of binding polynucleotides and magnetic beads capable of binding proteins has the advantage that preferred biological agents, e.g. a preselected biological agent, are bound to the beads capable of binding proteins via the specific antibody and that the preferred biological agent may thus be selectively isolated from the air sample. For example, selected biological agent(s) may be separated from biological agents and/or contaminants. Separated biological agents may then e.g. be lysed and released polynucleotides, e.g. DNA, may bind to magnetic beads capable of binding polynucleotides. The aforementioned preferred embodiments of the device using said mixture of magnetic beads may for example but not exclusively be used in the device illustrated in figures 3 and 4.

In one preferred embodiment the magnetic beads capable of binding polynucleotides are silica magnetic beads. Preferably, the beads are modified with sodium silica. If the magnetic beads comprise oligonucleotide primers on their surface, said oligonucleotide primers may be covalently or non-covalently linked to the surface of the beads. In a particularly preferred embodiment the oligonucleotide primers are biotinylated and the magnetic beads are coated with streptavidin. The oligonucleotide primers may then be attached to the surface of the beads by biotin-streptavidin binding.

Magnetic beads that comprise oligonucleotid primers on their surface provide the advantage that polynucleotides derived from a biological agent can hybridize to said oligonucleotide primers on the surface of the beads.

In a preferred embodiment said oligonucleotide primers are DNA oligonucleotides. Preferably the oligonucleotide primers have a length of about 5 to 100 nucleotides, preferably of about 10 to 50 nucleotides, most preferably of about 15 to 30 nucleotides.
In a preferred embodiment the oligonucleotide primers have their 5'-terminal ends attached to the substrate, such that their 3'-terminal ends are free to participate in primer extension reactions. The oligonucleotide primers can e.g. be attached directly to the coating of the bead via reactive groups or through a molecular spacer. Examples of reactive groups include amine, carboxylic acid, aldehyde, epoxy, cyanuric, hydroxy, hydrazide, carbodiimides, tosyl, thiol, and others. As spacers e.g. different molecular chains of Alcyclic-, Alcenyclic-, Alcinylic-, Heteroalcyclic-, Heteroalcenyclic- or Heteroalcinyclic origin may be used.

Preferably, the oligonucleotide primers are covalently attached to the beads. If the magnetic beads comprise oligonucleotide primers on their surface, it is preferred that one pool of said beads comprises forward primers for a target polynucleotide of interest on their surface and a second pool comprises reverse primers for the same target polynucleotide on their surface.

The terms "forward primer" and "reverse primer" are used herein according to their conventional and well known meaning in the art.

In some embodiments different types of magnetic beads capable of binding polynucleotides may be used, wherein the different types of magnetic beads comprise oligonucleotides primers specific for different target polynucleotides on their surface.

Magnetic beads as used herein preferably have a diameter of between 50 nm and 500 µm. In one preferred embodiment the beads have a diameter of between 100 nm and 100 µm, preferably of 500 nm and 50 µm, most preferably between 1 µm and 10 µm. In a preferred embodiment the magnetic beads in the collection chamber are repeatedly or constantly agitated to prevent them from sinking. The beads may e.g. be stirred in order to render binding of polynucleotides to the beads more efficient.
This process can for example be controlled by alternating magnetic fields generated by electro magnets placed in close proximity outside the liquid collection chamber. In a preferred embodiment the volume of the liquid collection chamber is connected to the volume of a magnetic bead reservoir. Preferably, said magnetic bead reservoir is a container in which magnetic beads are stored in a liquid as described above. Preferably said liquid is the same liquid that is used to collect particles from the air sample in (a). In a preferred embodiment samples of magnetic beads are transported from the magnetic bead reservoir to the liquid collection chamber at defined time intervals or continuously. Said transport of magnetic beads may e.g. be achieved by means of a magnet. Preferably said magnet is controlled automatically. It is further preferred that the magnetic beads are washed after having bound polynucleotides comprised in the liquid.

Therefore in a preferred embodiment the device according to the invention further comprises a magnetic bead washing unit, wherein beads received from the particle concentration unit are washed at least once by mixing the beads with a washing solution, temporarily immobilizing the magnetic beads on a magnet and replacing the washing solution with fresh washing solution while the beads are immobilized. Said washing unit preferably is a container whose volume is connected to the volume of the liquid collection chamber. The liquid collection unit and the washing unit may e.g. be connected by a channel or a tube. Magnetic beads may e.g. be transported from the liquid collection unit to the washing unit or vice versa by means of liquid flow and/or by means of a magnet that pulls the magnetic beads into the washing unit or into the liquid collection unit respectively.

The washing solution may be any water-based solution suitable for washing magnetic beads. In a preferred embodiment the washing solution is the same liquid that is used to collect particles from the air sample in (a). Prior to immobilizing the beads on a magnet, the beads may be stirred in order to achieve mixing with the washing solution.
The washing solution may be replaced by draining the washing unit and adding fresh washing solution to the washing unit. Preferably, the washing solution in the washing unit is replaced by an automated process.

In some preferred embodiments, washing of the beads may also be performed within the liquid collection chamber. In such a case washing may be performed as described above for the magnetic bead washing unit.

Preferably the beads are washed once, twice, three times or more than three times.

In a preferred embodiment the device according to the invention further comprises a dispensing unit which dispenses magnetic beads received from the liquid collection chamber to the amplification unit.

If the device according to the invention comprises a separate magnetic bead washing unit, the dispensing unit in one preferred embodiment may dispense magnetic beads received from the washing unit. Thus in one preferred embodiment the device according to the invention further comprises a dispensing unit which dispenses magnetic beads received from the liquid collection chamber or from a washing unit to the amplification unit.

In a preferred embodiment said dispensing unit is a container whose volume is connected to the volume of the liquid collection chamber and/or to the volume of the washing unit. The dispensing unit may be connected to the liquid collection chamber and/or to the washing unit by e.g. a channel or a tube. In some preferred embodiments the dispensing unit may be in the shape of a cylinder or a funnel.

In some further preferred embodiments the dispensing unit may comprise a pump, a plunger axially movable inside the dispensing unit, a delivery duct and/or a valve from which the magnetic beads are dispensed. In a preferred embodiment the dispensing unit at its outlet end comprises a valve that may be automatically opened and closed.
Therefore, the dispensing unit may comprise means to open and close the valve. Said means to open and close the valve may e.g. be electronic control means such as e.g. a microprocessor.

5 In a preferred embodiment the magnetic bead dispensing unit receives and dispenses beads at defined temporal intervals or continuously such that magnetic beads are dispensed at defined temporal intervals or continuously into the amplification unit. Preferably, the dispensing unit at each temporal interval dispenses a defined volume of magnetic beads into the amplification unit. If the amplification unit comprises PCR reaction containers, the dispensing unit preferably dispenses magnetic beads into the PCR reaction containers of the amplification unit.

As used herein the term "amplification unit" refers to a unit wherein the polynucleotides are amplified using polymerase chain reaction (PCR).

15 If the device according to the invention comprises a dispensing unit, the amplification unit preferably receives magnetic beads loaded with polynucleotides from the dispensing unit. In some other embodiments the amplification unit may also receive magnetic beads loaded with polynucleotides from the liquid collection chamber.

20 In a preferred embodiment the amplification unit allows for performing multiple amplification reactions in parallel. By "parallel" it is meant that different PCR reactions may be carried out at the same time. In one preferred embodiment each PCR reaction is started at a different time point. In such cases, the PCR reactions carried out in parallel will each be in a different stage of the PCR reaction at any given time point.

25 In one preferred embodiment the amplification unit of the device according to the invention is selected from the group consisting of:
(a) a rotatable disc comprising at least two PCR reaction containers and a heating device comprising different temperature zones;
(b) a channel suitable for transport of a PCR reaction mixture and a heating device comprising different temperature zones;
(c) an amplification unit that comprises different subunits, wherein each subunit comprises a reaction container and a heating device; and
(d) a microfluidic device comprising a microchannel suitable for transport of a PCR reaction mixture and a heating device comprising different temperature zones.

In a particularly preferred embodiment said rotatable disc in (a) comprises at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10 or more than 10 PCR reaction containers. Preferably, said PCR reaction containers are suitable for holding a PCR reaction mixture. For example, the PCR reaction containers may be wells. If the amplification unit comprises a rotatable disc comprising PCR reaction containers, it may e.g. allow for performing at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10 or more than 10 amplification reactions in parallel. In a particular preferred embodiment the amplification unit allows for performing 6 amplification reactions in parallel.

In a preferred embodiment the PCR reaction containers are filled with a PCR reaction mixture. Such PCR reaction mixtures are well known in the art and may e.g. comprise a PCR reaction buffer, a deoxynucleotide triphosphate (dNTP) mix, a DNA polymerase and an oligonucleotide primer pair.

In one preferred embodiment said DNA polymerase is a particularly heat stable DNA polymerase. One example of a suitable DNA polymerase is Taq polymerase. In some cases, for example when the polynucleotide derived from the biological agent is RNA, it is desirable to convert the RNA into cDNA prior to amplification.
Thus in one preferred embodiment the PCR reaction mixture further comprises a reverse transcriptase.

The oligonucleotide primer pair is preferably specific for a polynucleotide derived from a biological agent, e.g. the oligonucleotide primer pair may be specific for a polynucleotide derived from a pathogenic virus, a spore, a fungus, a parasite or a bacterium.

In one preferred embodiment each PCR reaction container comprises the same oligonucleotide primer pair. In a further preferred embodiment different PCR reaction containers comprises different oligonucleotide primer pairs.

For example, in cases where the amplification unit comprises 6 PCR reaction containers, each of the 6 PCR reaction containers may comprise the same oligonucleotide primer pair or each reaction container may comprise a different oligonucleotide primer pair. Alternatively, e.g. two of the PCR reaction containers may comprise a first oligonucleotide primer pair, two other PCR reaction containers may comprise a second oligonucleotide primer pair and two further PCR reaction containers may comprise a third oligonucleotide primer pair.

It is preferred that two or more PCR reaction containers comprise the same oligonucleotide primer pair. This provides the advantage that a given polynucleotide of interest when present in the air sample is amplified in more than one PCR reaction container, thus rendering detection of said polynucleotide more reliable.

In cases where different oligonucleotide primer pairs are present in different PCR reaction containers, more than one polynucleotide of interest can be detected in the air sample.

In a particularly preferred embodiment each PCR reaction container comprises only one oligonucleotide primer pair.
However, in some embodiments a PCR reaction container may also comprise more than one oligonucleotide primer pair (e.g. if a PCR multiplex reaction is to be performed). For example, a PCR reaction container may comprise 2, 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 oligonucleotide primer pairs.

In one preferred embodiment PCR is performed by exposing the PCR reaction containers comprising the amplification reaction and polynucleotides bound to the magnetic beads to different individual temperature zones.

It is preferred that the rotatable disc comprising said reaction containers is rotated over said heating device comprising different temperature zones.

The term "temperature zone" as used herein refers to different parts of the heating device, which may be adjusted to different individual temperatures. Preferably, the temperatures of the different temperature zones of the heating device are individually controllable. The temperature of a temperature zone may be adjusted by heating or cooling. The temperature in each temperature zone may e.g. be adjustable to a value between 0° and 98° C. In a particularly preferred embodiment the temperature in each temperature zone is kept constant. Adjusting and/or maintaining the temperature in each temperature zone may e.g. be achieved by one or more temperature controllers, comprising e.g. heaters, coolers and/or heat transfer means. If the amplification unit is a rotatable disc, it is preferred that the heating device is disc-shaped. The heating device may e.g. comprise 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 different temperature zones. In a preferred embodiment the heating device comprises 3 different temperature zones. It is preferred that the temperature in one of the temperature zones is adjusted to a value suitable for primer annealing to a target polynucleotide (annealing zone), the temperature in the second zone is adjusted to a value suitable for primer extension (extension zone) and the temperature in the third is adjusted to a value suitable for denaturation of the target polynucleotide (denaturation zone).
The temperature in the annealing zone may e.g. be adjusted to a value from about 40°C to about 75°C, preferably from about 50°C to about 70°C. The temperature in the extension zone may e.g. be adjusted to a value from about 70°C to 80°C, preferably about 72°C to 75°C. The temperature in the denaturation zone may e.g. be adjusted to a value from about 90°C to about 98°C. In a particular preferred embodiment the temperature in the annealing zone is adjusted to 60°C, the temperature in the extension zone is adjusted to 72°C and the temperature in the denaturation zone is adjusted to 95°C.

In cases where reverse transcription is to be performed prior to the amplification reaction, the temperature in one or more than one of the temperature zones may be adjusted to a value from about 45°C to 65°C, preferably to a temperature of about 50°C prior to amplification in order to allow reverse transcription to take place. In such cases the temperature in the temperature zones are preferably adjusted to annealing temperature, extension temperature or denaturation temperature after finalizing reverse transcription. Alternatively, the heating surface in addition to the temperature zones used for amplification of polynucleotides may comprise individual temperature zones that are constantly kept at 45°C to 65°C, preferably at a temperature of about 50°C, in order to allow for reverse transcription of RNA templates.

It is preferred that the reaction containers of the rotatable disc comprising the amplification reaction and the target polynucleotides bound to the magnetic beads are consecutively exposed to the different temperature zones in order to amplify the target polynucleotide. In a preferred embodiment the reaction containers are exposed to the different temperature zones by rotating the rotatable discover said heating device.

Preferably, said reaction containers are consecutively exposed to the annealing temperature, the extension temperature and the denaturation temperature.
In one preferred embodiment the reaction containers are exposed to each temperature several times in a cyclic way, i.e. the reaction containers are repeatedly exposed to the annealing temperature, the extension temperature and the denaturation temperature, wherein one passage through the 3 temperatures constitutes one temperature cycle, as is routinely practiced when carrying out a standard PCR reaction as known in the art. It is preferred that multiple temperature cycles are performed. In a preferred embodiment, between 20-60 more preferably between 25-50, even more preferably between 30-40 temperature cycles are performed. In some embodiments fresh PCR-reaction mix may be continuously added to the reaction containers, while the magnetic beads loaded with polynucleotides are added to the solution.

If a target polynucleotide of interest is present in the air sample, it will be amplified in the described PCR reaction.

As described above in (b), in a further preferred embodiment the amplification unit comprises a heating device comprising different temperature zones and a channel suitable for transport of a PCR reaction mixture.

In a preferred embodiment said heating device is disc-shaped and the channel for transport of a PCR reaction mixture is circular. In a preferred embodiment, the channel passes through the different temperature zones of the heating device. Preferably, the temperatures of the different temperature zones of the heating device are individually controllable as already described above.

Preferably, the channel contains a PCR reaction mixture comprising e.g. a PCR reaction buffer, a deoxynucleotide triphosphate (dNTP) mix and a DNA polymerase. Preferably the PCR reaction mixture may further comprise a reverse transcriptase. It is preferred that the PCR reaction mixture does not comprise an oligonucleotide primer pair if the PCR is performed on the beads as described below.
However, in some embodiments oligonucleotide primers bound to magnetic beads and oligonucleotide primers which are free in solution can be combined in one reaction, increasing the elongation efficiency at the oligonucleotide primers attached to the beads.

In a preferred embodiment the channel receives magnetic particles loaded with polynucleotides from the liquid collection chamber or from a magnetic bead washing unit. In a more preferred embodiment the channel receives magnetic beads loaded with polynucleotides from a dispensing unit.

If the device according to the invention comprises the afore-described amplification unit, it is preferred that the PCR reaction takes place on the beads (PCR-on-the-beads).

For said purpose the magnetic beads preferably comprise oligonucleotide primers on their surface and the target polynucleotides are hybridized to said oligonucleotide primers on the beads. It is further preferred that one pool of said beads comprise forward oligonucleotide primers on their surface and that another pool of beads comprises reverse oligonucleotide primers on their surface. As both the forward and the reverse primers are bound to magnetic beads, extension of target polynucleotides will take place on the beads.

For amplification of target polynucleotides, the magnetic beads are preferably transported within the channel through the different temperature zones of the amplification unit in a circular manner by means of a magnet.

In one preferred embodiment in addition to the magnetic beads also the PCR reaction mixture is transported through the different temperature zones in a circular manner. Preferably, the PCR reaction mixture in the channel is differentially heated in the different temperature zones of the heating device.
Preferably, the magnetic beads are repeatedly transported through an annealing zone, an extension zone and a denaturation zone in a circular manner. In another preferred embodiment, the magnetic beads are repeatedly transported through a denaturation zone, an extension zone and a hybridization zone in a circular manner. It is preferred that multiple temperature cycles are performed. In a preferred embodiment, between 20-60, more preferably between 25-50, even more preferably between 30-40 temperature cycles are performed.

As outlined above in (c), in yet another preferred embodiment the amplification unit comprises different subunits, wherein each subunit comprises a reaction container and a heating device. Preferably each of said heating devices comprises only one temperature zone, i.e. each heating device is heated to only one temperature. Preferably said temperature is constant. In a preferred embodiment each reaction container comprises a liquid, preferably a PCR reaction mixture comprising e.g. a PCR reaction buffer, a deoxynucleotide triphosphate (dNTP) mix and a DNA polymerase. Preferably the PCR reaction mixture may further comprise a reverse transcriptase. It is preferred that the PCR reaction mixture does not comprise a primer pair if the PCR is performed on the beads. However, in some embodiments oligonucleotide primers bound to magnetic beads and oligonucleotide primers which are free in solution can be combined in one reaction.

In a further preferred embodiment the reaction containers of the different subunits are connected by e.g. a channel or a tube.

In another preferred embodiment one of the reaction containers of the different subunits of the amplification unit is connected to the liquid collection chamber. In one preferred embodiment, the amplification unit comprises 1,2,3,4,5,6,7,8,9,10 or more than 10 different subunits.
In a particularly preferred embodiment the amplification unit comprises 3 subunits. It is preferred that the heating device of each subunit is adjusted to a different temperature.

The temperature of each heating device may e.g. be adjustable to a value between 0°C and 98°C. It is preferred that the temperature of one of the heating devices is adjusted to a value suitable for denaturation of the target polynucleotide (denaturation temperature), the temperature of a second heating device is adjusted to a temperature suitable for primer annealing to a target polynucleotide (annealing temperature) and the temperature of a third is adjusted to a value suitable for primer extension (extension temperature). In another preferred embodiment, the temperature of one of the heating devices is adjusted to denaturation temperature, the temperature of a second heating device is adjusted to extension temperature and the temperature of a third heating device is adjusted to hybridization temperature, i.e. a temperature of e.g. about 40°C to about 75°C.

Preferably, the temperature of the liquid in each reaction container is adjusted by the corresponding heating device. In a preferred embodiment the subunits are arranged in a consecutive manner.

In one embodiment magnetic beads loaded with polynucleotides are transported from the liquid collection chamber into the first subunit of the amplification unit (denaturation temperature) and then into the second subunit (annealing temperature) and third subunit (extension temperature). In another embodiment magnetic beads loaded with polynucleotides are transported from the liquid collection chamber into the first subunit of the amplification unit (denaturation temperature) and then into the second subunit (extension temperature) and third subunit (hybridization temperature).
The magnetic beads are preferably transported by means of a magnet. Preferably, after reaching the third subunit the magnetic beads are transported back to the liquid collection chamber. In a preferred embodiment, the magnetic beads are repeatedly transported in a back-and-forth manner.

It is preferred that between 20-60, more preferably between 25-50, even more preferably between 30-40 cycles are performed. One "cycle" is this context refers to one step of transporting the magnetic beads through the subunits of the amplification unit and back into the liquid collection chamber. It is preferred that the temperature in the first subunit of the amplification unit is only adjusted to denaturation temperature after the first cycle is completed. It is preferred that in the first cycle the temperature in the first subunit is adjusted to a temperature below denaturation temperature, e.g. to a temperature between 0°C and 60°C.

If the device according to the invention comprises the afore-described amplification unit, it is preferred that the PCR reaction takes place on the beads (PCR-on-the-beads).

For said purpose the magnetic beads preferably comprise oligonucleotide primers and extension of target polynucleotides preferably takes place on the beads as outlined above.

As described above in (d), in a further preferred embodiment the amplification unit comprises a microfluidic device comprising a microchannel suitable for transport of a PCR reaction mixture and a heating device comprising different temperature zones. In a preferred embodiment the microfluidic device is a PCR microfluidic chip. Said chip may e.g. be a silicon or glass chip. In a preferred embodiment the chip is a polycarbonate chip.
Preferably, said heating device comprises at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten or more than ten temperature zones. In a particularly preferred embodiment the heating device comprises three temperature zones. Preferably, one of the temperature zones is adjusted to denaturation temperature, a second temperature zone is adjusted to annealing temperature and a third temperature is adjusted to extension temperature. In a preferred embodiment the PCR reaction mixture within the microchannel is differentially heated by the different temperature zones of the heating device. For example, the chip may be placed onto said temperature zones in such a way that the microchannel passes by the different temperature zones of the heating device. In one preferred embodiment, said microchannel repeatedly passes by different temperature zones of the heating device in a meandering pattern.

It is preferred that the microchannel is suitable for transport of a PCR reaction mixture and for transport of magnetic beads loaded with polynucleotides. An example of a suitable microfluidic device and its use is e.g. described in "Methods and instruments for continuous-flow PCR on a chip", C. Gartner, R. Klemm, H. Becker, Proc. "Microfluidics, BioMEMS and Medical Microsystems V", San Jose, SPIE Vol. 6465, SPIE 2007, 6465502-1 - 6465502-8, which is hereby incorporated by reference.

In order to detect harmful biological agents as early as possible, it is desirable to monitor the amount of a polynucleotide of interest while it is amplified, i.e. the increase in the amount of said polynucleotide is preferably measured in "real time". Therefore, in a preferred embodiment the amplification unit is suitable for performing real-time PCR.

In one preferred embodiment the PCR reaction mixture used in the amplification unit comprises a DNA-binding dye capable of binding to double stranded DNA, such as e.g. SYBR green or ethidium bromide.
In a particular preferred embodiment the PCR reaction mixture comprises fluorescent hybridization probes. Preferably said probes are labeled with a fluorescent dye and a quencher dye. Examples of such probes are e.g. molecular beacons, TaqMan probes or scorpion primers.

Such probes provide the advantage that they allow multiple DNA species to be measured in the same sample (multiplex PCR), since fluorescent dyes with different emission spectra may be attached to the different probes.

The aforementioned hybridization probes are well known in the art and the skilled person knows how to design and use such probes.

In a preferred embodiment the "detection system" of the device of the invention detects polynucleotides amplified in said amplification unit in real-time. "Real time detection" may include detection of the kinetic production of signal, comprising taking a plurality of measurements in order to characterize the signal over a period of time. The fluorescence intensity for each amplification reaction may be determined using a detector, such as a charge-coupled device (i.e. CCD camera) or other suitable instrument capable of detecting the emission spectra for the fluorescent dyes of molecules used.

In a preferred embodiment fluorescence is excited by laser or halogen light and is detected by a charge-coupled device (CCD) camera.

A computer may be used for data collection and processing. Data processing may be achieved by using a suitable imaging software.
For further details on real-time PCR methodology and signal detection and quantification, reference can e.g. also be made to Dorak, M. Tevfik (ed.), Real-Time PCR, April 2006, Taylor & Francis; Routledge, 978-0-415-37734-8.

In an alternative embodiment the elongated double DNA strand attached to the bead can be denatured by heat and the resulting single DNA strands can be separated by magnetic force and hybridized to specific corresponding oligonucleotides bound to a microarray, which is placed inside the detection chamber.

For the microarray different sensor technologies can be used to read out the signal, such as e.g. optical readout (e.g. fluorescence or color dyes), electrochemical readout, magneto resistive readout (e.g. based on AMR, GMR, TMR effects), surface acoustic waves or impedance amongst others.

In a preferred embodiment the device according to the invention further comprises a control unit which provides overall automated control of the device according to the invention. Preferably, said control unit comprises a computer.

It is preferred that the control unit further comprises the function of a data processing and transmission unit. Thus, in a further preferred embodiment said control unit further communicates the data received from the PCR reactions to a predetermined location, such as e.g. a central military unit. The data may e.g. be transmitted via radio-communication or via satellite. The data may then be correlated via global positioning system (GPS) with the area where the air sample was derived from. This allows for quick identification of a contaminated area. In order to provide secure transmission of data, encryption technology may be used to encrypt the data before transmission from the detection unit to the receiving ground unit, where the data are being decoded.
The present invention in a further aspect relates to a method for detecting biological agents in an air sample comprising at least the steps of:

(a) providing an air sample;
(b) collecting particles from the air sample into a liquid;
(c) releasing polynucleotides from the particles into the liquid
(d) binding the released polynucleotides to magnetic beads
(e) subjecting the polynucleotides captured on the magnetic beads to polymerase chain reaction; and
(f) detecting the amplified polynucleotides.

In a preferred embodiment the method further comprises the step of processing the obtained data and identifying biological agent(s) present in the air sample.

In another preferred embodiment the method further comprises the step of data encryption and transmission from the detecting unit to a ground unit.

In a preferred embodiment of the method, the steps (a), (b), (c), (d), (e) and (f) are carried out in that order, wherein preferred additional steps may be carried out between steps (a), (b), (c), (d), (e) and (f) as is further specified below.

An air sample may be provided and particles from said air sample may be collected into a liquid as described above in the context of the device of the invention. Methods to release polynucleotides from biological agents have been described above in detail and may e.g. include lysing the particles collected in (b) by means of heat, treatment with ultra-sound and/or treatment with a detergent.

The magnetic beads that have been described herein above in the context of the device of the invention can also be used in the method of the invention. In one preferred embodiment, in step (d), said released polynucleotides are preferably bound to the magnetic beads at a controlled temperature.
Preferably said temperature is selected from a temperature between room temperature and 72°C. If the released polynucleotides specifically hybridize to oligonucleotides on the surface of the magnetic beads as also described above, then various hybridization buffers can be used that are well known in the art. For example, the hybridization may be performed in TE-buffer or in PCR-buffer.

In a preferred embodiment of the method, following step (d) at least one washing step is carried out which comprises temporarily immobilizing the magnetic beads on a magnet, preferably an electro-magnet, and replacing the liquid in which the beads are suspended with fresh liquid while the beads are immobilized.

In a preferred embodiment at least two, at least three or more than three washing steps are carried out. Suitable washing buffers are known to the skilled person. In one preferred embodiment the same buffer may be used for washing the beads that has been used for lysing the particles in step (c).

The advantage of said washing step is that following lysis, non-polynucleotide molecules such as proteins, dust and other material can efficiently be removed from the magnetic beads prior to carrying out the PCR reaction in step (e). In a particularly preferred embodiment of the method, steps (a) through (d) and said washing step are carried out two or more times in that order to effectively remove unwanted molecules.

Repeating these steps provides the further advantage that polynucleotides become effectively enriched on the magnetic beads.

Once particles from an air sample volume have been collected, lysed and the polynucleotides released have bound to said magnetic beads, polynucleotides captured on the magnetic beads are subjected to PCR in step (e).
For this purpose said magnetic beads are preferably transferred to an amplification unit where the beads are contacted with a suitable PCR reaction mixture. Said PCR reaction mixture may e.g. comprise a PCR reaction buffer, a dNTP mix, a DNA polymerase and optionally a reverse transcriptase. If the forward and the reverse primer for a target polynucleotide of interest are bound to different pools of magnetic beads such that extension of target polynucleotides takes place on the beads as outlined above in the context of the device of the invention, it is in some embodiments, as e.g. the embodiments outlined above in the context of the device according to the invention, preferred that the PCR reaction mixture does not contain any additional oligonucleotide primers in solution. However, in some embodiments oligonucleotide primers bound to magnetic beads and oligonucleotide primers which are free in solution can be combined in one reaction.

In one preferred embodiment the polymerase chain reaction is a real-time PCR reaction. Real-time PCR and detection of the amplified polynucleotides may be carried out as described above in the context of the device of the invention.

The method according to the invention is suitable for sampling individual volumes of air and detecting biological agents comprised therein by analyzing polynucleotides released from said biological agents using real time PCR. It is preferred that steps (a) through (f), optionally including the above-outlined washing step(s), are repeated multiple times, preferably continuously. This allows for a repeated and preferably continuous analysis of air and thus a harmful biological agent can be detected in nearly real-time.

In a preferred embodiment steps (a) to (f) of the method according to the invention are repeated at least once.

In a particularly preferred embodiment steps (a) to (f) of the method according to the invention and optionally the washing step are repeated at least twice, at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times, at
least 9 times, at least 10 times, at least 12 times, at least 13 times, at least 20 times of more than 20 times. In a particularly preferred embodiment steps (a) to (f) are repeated continuously over a given period of time. A repetition of the sequence of steps (a) to (f) may in some embodiments already start before the previous sequence has been finished. For example, a repetition of steps (a) to (f) may be started every 5 minutes over a time period of 30 minutes. If the method according to the invention comprises steps in addition to steps (a) to (f), said steps may also be repeated.

It is also within the ambit of the invention that multiple instances of the method are carried out in parallel which further improves the analysis rate. For example, two devices carrying out the method could work in parallel wherein the collected particles are used to start individual PCR reactions, wherein in each PCR reaction a respective batch of magnetic beads is analyzed. In one preferred embodiment the device according to the invention is suitable for performing the method according to the invention.
CLAIMS

1. A device for detecting a biological agent in an air sample, the device comprising:
   (a) a particle concentration unit that collects particles from the air sample into a liquid;
   (b) a liquid collection chamber comprising magnetic beads, wherein the magnetic beads are capable of binding polynucleotides;
   (c) an amplification unit suitable for amplifying polynucleotides bound to the magnetic beads; and
   (d) a detection system for detecting amplified polynucleotides.

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

3. A device according to any of claims 1 or 2, wherein the amplification unit allows for performing multiple amplification reactions in parallel.

4. A device according to any of claims 1 to 3, wherein the amplification unit is selected from the group consisting of:
   (a) a rotatable disc comprising at least two PCR reaction containers and a heating device comprising different temperature zones;
   (b) a channel suitable for transport of a PCR reaction mixture and a heating device comprising different temperature zones;
   (c) an amplification unit that comprises different subunits, wherein each subunit comprises a reaction container and a heating device; and
   (d) a microfluidic device comprising a microchannel suitable for transport of a PCR reaction mixture and a heating device comprising different temperature zones.
5. A device according to claim 4, wherein the temperatures of the different temperature zones of the heating device are individually controllable.

6. A device according to any of claims 1 to 5, wherein the amplification unit is suitable for performing real-time PCR.

7. A device according to any of claims 1 to 6, wherein the device further comprises a dispensing unit which dispenses magnetic beads received from the liquid collection chamber to the amplification unit.

8. A device according to claim 7, wherein the dispensing unit receives and dispenses beads at defined temporal intervals or continuously such that magnetic beads are dispensed at defined temporal intervals or continuously into the amplification unit.

9. A device according to any of claims 1 to 8, wherein the device further comprises a magnetic bead washing unit.

10. A device according to any of claims 1 to 9, wherein the magnetic beads
   (i) are positively charged;
   (ii) comprise oligonucleotide primers on their surface capable of specifically hybridizing to target polynucleotides derived from a biological agent; and/or
   (iii) comprise basic functional groups that have been covalently linked to the bead surface.

11. A device according to any of claims 1 to 10, wherein the magnetic beads in the liquid collection chamber are repeatedly or constantly agitated to prevent them from sinking.
12. A method for detecting biological agents in an air sample comprising at least the steps of:
   (a) providing an air sample;
   (b) collecting particles from the air sample into a liquid;
   (c) releasing polynucleotides from the particles into the liquid;
   (d) binding the released polynucleotides to magnetic beads capable of binding said polynucleotides;
   (e) subjecting the polynucleotides captured on the magnetic beads to polymerase chain reaction; and
   (f) detecting and/or quantifying the amplified polynucleotides.

13. A method according to claim 12, wherein following step (d) at least one washing step is carried out which comprises temporarily immobilizing the magnetic beads on a magnet and replacing the liquid with fresh liquid while the beads are immobilized.

14. A method according to claim 12 or 13, wherein steps (a) to (f) and optionally the washing step according to claim 13 are repeated at least once.

15. A method according to any of claims 12 to 14, wherein the polymerase chain reaction is a real-time PCR reaction.
Fig. 2

Top-View:
- Particle concentration unit and liquid collection chamber unit optionally.
- Dispensing unit
- Optional waste container

Diagram:
- Heating device comprising different temperature zones (here: three temperature zones shown)
- Preferred: insulation between temperature zones
- Rotatable dish
- PCR reaction container (preferably two or more per temperature zone)
- Electro-magnet
- Air sample comprising biological agent(s)
Fig. 5

- Top View:
  - Particle concentration unit
  - Liquid collection chamber
  - Optionally, dispensing unit

- Mechanical device comprising:
  - Heating device comprising different temperature zones (three temperature zones shown)
  - Electro magnet

- Magnetic beads

- Detector of detection system of amplification unit
A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68 G01N35/00 G01N33/543
ADD.

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)
C12Q G01N

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
</tr>
</thead>
</table>

X Further documents are listed in the continuation of Box C

D 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 document 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

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

& document member of the same patent family

Date of the actual completion of the international search
17 September 2010

Date of mailing of the international search report
04/10/2010

Name and mailing address of the ISA
European Patent Office, P B 5818 Patentilaan 2 NL- 2280 HV Rijswijk Tel (+31-70) 340-2040, Fax (+31-70) 340-3016

Authorized officer
Leber, Thomas
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category</td>
<td>Citation</td>
<td>Relevant to claim No.</td>
</tr>
<tr>
<td>----------</td>
<td>---------------------------------------------------------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>A</td>
<td>ROBERT S DUNGAN ET AL: &quot;Qualitative and quantitative methodologies for determination of airborne microorganisms at concentrated animal-feeding operations&quot; WORLD JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY, KLUWER ACADEMIC PUBLISHERS, DOI: 10.1007/S11274-009-0043-1, vol. 25, no. 9, 26 April 2009 (2009-04-26), pages 1505-1518, XP019734632 ISSN: 1573-0972 the whole document</td>
<td>1-15</td>
</tr>
</tbody>
</table>