Title: AIR SAMPLING DEVICE

Abstract: Described is an air sampling device which comprises a body section, an inlet for allowing air to enter the body section, a first outlet for allowing air to exit the body section towards a collection vessel; and a second outlet for allowing air to exit the body section towards a vacuum pump. The inlet, first outlet and second outlet are configured such that when the second outlet is connected to a vacuum pump, a major air flow path is created between the inlet and the second outlet and a minor air flow path is created between the inlet and the first outlet, and airborne particles which enter the device through the inlet follow either the major air flow path or the minor air flow path according to their size. Also described is an apparatus and an automated method for detecting airborne biological particles.

Fig 1

45.5mm 0/0
x 39.5mm 1/0
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1

AIR SAMPLING DEVICE

The present invention relates to air sampling devices, in particular to air sampling devices for use in detection of airborne biological particles.

In various situations such as those typical to the farming, domestic, clean-room, food and feed industries and in environments such as the battlefield, airports, ports, or in clinical settings, it is desirable to know whether certain airborne biological particles are present. It is of great use to know whether particles, such as human or plant pathogens, food spoilage organisms or allergens such as fungal spores, plant spores, pollens, or bacteria, GM pollen, GMOs, fungicide resistant genets of a fungus, or antibiotic resistant genets of a bacterium, are present in order to guide decisions on mitigation of their effects. The effects of such biological particles include human or plant disease, allergy or contamination.

Whilst air sampling devices are known, they suffer from a number of drawbacks. In particular, there is a need for stand-alone air sampling devices that can be left in the field with minimal user input. Although stand-alone air sampling devices are available, they are not conducive to further automation to allow processing of the samples collected.

For example, it is known to provide a multi-vial cyclone sampler for the detection of airborne spore and particle matter. For example, the multi-vial cyclone device made by Burkard Manufacturing Co. Ltd or a miniature wet cyclone device, such as the Coriolis sampler made by Bertin Technologies. For the multivial cyclone, samples are collected into 1.5ml collection vials located on a carousel. Movement of the carousel and collection vials can be operated by external switching or remote control. However, the nature of the cyclone sampler means that air is drawn into the collection vials, which will result in rapid evaporation of any liquid present in the collection vial. Therefore, multi-vial cyclone samplers are not compatible with methods which require the collection of airborne particles into liquid culture media or reagents over an extended time period. The speed and accuracy of testing using a multi-vial cyclone sampler is also limited by the need for further analysis to be conducted separately from the device by a person skilled in such analysis. Without the possibility of automating further analysis, such devices are not suited for use by a non-specialist user, such as a farmer.
It is, therefore, an object of the present invention to seek to alleviate the above identified problems.

SUMMARY OF THE INVENTION

According to one aspect of the present invention, there is provided an air sampling device which comprises:-

(i) a body section;
(ii) an inlet for allowing air to enter the body section;
(iii) a first outlet for allowing air to exit the body section towards a collection vessel; and
(iv) a second outlet for allowing air to exit the body section towards a vacuum pump,

the inlet, first outlet and second outlet being configured such that when the second outlet is connected to a vacuum pump, a major air flow path is created between the inlet and the second outlet and a minor air flow path is created between the inlet and the first outlet, and airborne particles which enter the device through the inlet follow either the major air flow path or the minor air flow path according to their size,

the inlet comprising an inlet passage tapered from an inlet passage first end having an inlet passage first diameter to an inlet passage second end having an inlet passage second diameter, the inlet passage first diameter being greater than the inlet passage second diameter,

the first outlet comprising a first outlet passage tapered from a first outlet passage first end having a first outlet passage first diameter to a first outlet passage second end having a first outlet passage second diameter, the first outlet passage first diameter being less than the first outlet passage second diameter,

the passages of the inlet and first outlet being substantially in alignment to form a minor air flow path between the inlet first end and the first outlet second end,

the inlet second end and first outlet first end being separated by a separation gap,

the second outlet being positioned for drawing air through the separation gap from the minor air flow path along a major air flow path formed between the inlet first end and a vacuum pump connected to the second outlet,
wherein the inlet passage first diameter is between about 1.3 cm and about 1.9 cm, the inlet passage second diameter is between about 0.2 cm and about 0.8 cm, the first outlet passage first diameter is between about 0.2 cm and about 0.8 cm, the first outlet passage second diameter is between about 0.6 cm and about 1.2 cm.

Preferably, the inlet passage second diameter and first outlet passage first diameter are about equal. Alternatively, the first outlet passage first diameter is greater than the inlet passage second diameter.

Preferably, the inlet passage first diameter is between about 1.5 cm and about 1.7 cm, preferably about 1.6 cm.

Preferably, the inlet passage second diameter is between about 0.4 cm and about 0.6 cm, preferably about 0.5 cm.

Preferably, the inlet passage second diameter is between about 0.3 cm and about 0.6 cm, preferably about 0.3 cm or about 0.4 cm.

Preferably, the first outlet passage first diameter is between about 0.4 cm and about 0.6 cm, preferably about 0.5 cm.

Preferably, the first outlet passage first diameter is between about 0.5 cm and about 0.7 cm, preferably about 0.6 cm.

Preferably, the first outlet passage second diameter is between about 0.8 cm and about 1.0 cm, preferably about 0.9 cm, most preferably 0.89 cm.

Preferably, the first outlet passage second diameter is between about 0.9 cm and about 1.1 cm, preferably about 1.0 cm.

Preferably, the inlet passage second diameter is about 0.4 cm and the first outlet passage first diameter is about 0.6 cm.
Preferably, the inlet passage second diameter is about 0.3 cm, the first outlet passage first diameter is about 0.6 cm and the first outlet passage second diameter is about 1.0 cm.

Preferably, the inlet passage is between about 3.0 cm and about 3.5 cm in length, preferably between about 3.2 cm and about 3.3 cm, preferably about 3.25 cm, most preferably 3.23 cm.

Preferably, the first outlet passage is between about 1.8 cm and about 2.3 cm in length, preferably between about 2.0 cm and about 2.1 cm, preferably about 2.05 cm, most preferably 2.04 cm.

Preferably, the separation gap is adjustable.

Preferably, the separation gap is measured from zero (fully closed) to a chosen gap size.

Preferably, the separation gap is a vertical distance between the inlet second end and first outlet first end.

Preferably, the separation gap is less than about 2mm, preferably less than about 1mm, preferably less than about 0.5mm, preferably less than about 0.25mm, preferably less than about 0.1mm. In some embodiments, the separation gap is about 0.76mm.

Preferably, the separation gap is at least about 0.6mm.

Preferably, the separation gap is between about 0.6mm and about 2mm.

Preferably, the inlet passage second end is located within the first outlet passage first end.

Preferably, the major airflow path is non-linear and the minor airflow path is substantially linear, such that heavier airborne particles will follow the minor airflow path and lighter airborne particles will follow the major airflow path.

Preferably, the inlet and first outlet are at opposing ends of the air sampling device.
Preferably, the second outlet is positioned between the inlet and the first outlet, preferably extending from a side wall of the air sampling device.

Preferably, the air sampling device comprises a means for directing the inlet into the path of a wind.

Preferably, the air sampling device comprises one or more baffle plates, for example a wind vane.

More preferably, the air sampling device comprises a rotatable means for directing the inlet into the path of the wind.

Preferably, the air sampling device comprises a vacuum pump connected to the second outlet.

Preferably, the air sampling device is configured to operate at an airflow rate of between about 15 L/min and about 30 L/min, preferably between about 20 L/min and about 25 L/min, most preferably at about 20L/min or about 23 L/min.

Preferably, the air sampling device has a width of less than about 8 cm.

Preferably, the air sampling device has a depth of less than about 8 cm.

Preferably, the air sampling device has a height of less than about 8 cm.

Preferably, the width is between about 4 cm and about 8 cm, preferably between about 5 cm and about 7 cm, preferably about 6.5 cm, most preferably 6.35 cm.

Preferably, the depth is between about 4 cm and about 8 cm, preferably between about 5 cm and about 7 cm, preferably about 6.5 cm, most preferably 6.35 cm.

Preferably, the height is between about 3 cm and about 7 cm, preferably between about 4 cm and about 6 cm, preferably about 5 cm.
Remarkably, the present invention provides an airborne biological particle sampler comprising a volumetric virtual impactor, with efficient collection capability having the ability to deliver two to three-fold more airborne particles with aerodynamic diameter 30 µm into a separate collection vessel compared to the current commercial equivalent product, the multivial cyclone (Burkard Manufacturing Co. Ltd).

The collection vessel of the present invention also minimises air flow at the base of the device such that the vessel can contain liquid. Any such liquid reagents are less prone to evaporation than liquids exposed to airflows necessary for direct impaction. The vessel can therefore contain liquid growth medium or liquid nucleic acid extraction buffer or any other liquid as determined by the user at ambient temperature, to allow for further processing and improved detection of particles.

According to another aspect of the present invention, there is provided an apparatus for detecting airborne biological particles, the apparatus comprising: -

(i) a virtual impactor,
(ii) one or more collection vessels for collecting biological particles which pass through the virtual impactor,
(iii) one or more detection means for detecting biological particles within the one or more collection vessels.

Preferably, the virtual impactor is an air sampling device as described herein.

Preferably, the one or more detection means permit detection of biological particles by chemical, immunological or nucleic acid based detection. For example, the detection may be of a specific DNA sequence and recorded by measurement of colour or fluorescence.

Preferably, the apparatus further comprises an output means for transmitting an output signal, for example a detection signal, from the detection means to a user. For example, the output could be in the form of an electronic signal.
Preferably, the output means transmits a detection signal to a user wirelessly, for example by GSM text.

Preferably, the virtual impactor is rotatable for positioning an inlet of the virtual impactor into alignment with wind direction.

Preferably, the one or more collection vessels contain reagents for use by the one or more detection means. For example, the one or more collection vessels may contain liquid growth media or liquid nucleic acid extraction buffer.

Preferably, the one or more collection vessels contain reagents for facilitating the release of cell contents.

Preferably, the apparatus comprises means for adding reagents to the one or more collection vessels, for example after collection of biological particles from the virtual impactor.

Preferably, the apparatus comprises collection vessel conveying means for moving the one or more collection vessels from a pre-collection position to a collecting position, and from a collecting position to a post-collecting position. Preferably, the conveying means comprises one or more carousels and/or one or more conveyor belts.

Preferably, the apparatus is controlled by a computer.

Preferably, the apparatus is configured to collect air samples for set time periods.

According to another aspect of the present invention, there is provided an automated method for detecting airborne biological particles, the method comprising use of an air sampling device or apparatus as described herein.

Preferably, the method comprises obtaining a sample of air, analysing the sample of air for the presence of biological particles, outputting a signal corresponding to the presence or
absence of biological particles to a user, preferably via wireless communication, for example via GSM text or email.

Preferably, the signal provides information regarding the amount and identity of the biological particles.

Preferably, the method comprises extracting DNA and/or a metabolite from biological particles in the sample of air, detecting DNA of a specific sequence and/or a target metabolite, and outputting a signal corresponding to the presence or absence of the DNA or metabolite.

The present invention provides an airborne biological particle sampler comprising a volumetric virtual impactor, able to deliver two to three-fold more airborne particles with aerodynamic diameter greater than 30 µm into a separate collection vessel compared to the current commercial equivalent product, the multivial cyclone (Burkard Manufacturing Co. Ltd).

Preferably, the operation of the sampler can be programmed to collect a sample for a user-defined set period. Preferably, an automated system is included to facilitate transferring the collected sample in the collection vessel to downstream processing steps aimed at automated detection of specific biological particles and provision of a new collection vessel for the next sampling period. The action of the virtual impactor minimises air transfer at the base of the collection vessel, hence the collection vessel can contain liquid, which will be less prone to evaporation than if exposed to direct impaction. Preferably, the vessel contains liquid growth medium or liquid nucleic acid extraction buffer as determined by the user and an automated processing system used to facilitate detection of one or many specific molecules by specific chemical reaction, nucleic acid primers or antibodies, followed by translation of the assay(s) into an electronic signal or signals communicated by GSM text or wired system (within 2 hours of the end of air sample collection in the case of DNA-based detection).

Automated post-sampling steps can be tailored to different user-defined detection methods including steps to incubate the sample at a user-defined temperature to facilitate growth of
viable cells or extraction of metabolites, proteins, DNA or other nucleic acids. Automated steps enable the collected, incubated contents to be tested for presence of chemical metabolites or to be transferred to a new vessel via a filtering stage, and, or new reagents added to facilitate detection of one or many specific molecules by specific nucleic acid primers or antibodies, followed by translation of the assay(s) into an electronic signal or signals communicated by GSM text or wired system. For nucleic acid based detection methods, results will be communicated within 2 hours of the end of air sample collection.

A number of technical difficulties faced the inventors when implementing the present invention. For example, it is necessary for the air sampling device and apparatus of the invention to be small enough to be field portable and to have sufficiently low power consumption to run from a 12v battery supply. Remarkably, despite these technical challenges, the collection efficiency of the present invention is very high and enables sampling into a collection liquid for much longer periods than that of other devices. For example, the Coriолос sampler, previously referred to, enables sampling for up to 30 minutes. The present invention allows sampling into liquid for about 12 hours. Furthermore, the present invention allows for very high collection efficiency, with approximately 70% of particles collected compared to those collected with a 2mm x 14mm cascade impactor (identical to a Hirst-type spore trap) when those particles had an aerodynamic diameter of 4.8 μm and over 3.5-fold collected with aerodynamic diameter 30-33 μm compared to the number collected by a Burkard multivivial cyclone sampler.

Within this specification embodiments have been described in a way which enables a clear and concise specification to be written, but it is intended and will be appreciated that embodiments may be variously combined or separated without parting from the invention.

DETAILED DESCRIPTION

Example embodiments of the present invention will now be described with reference to the accompanying figures, in which:-
Figure 1 shows a cross-sectional view of an air sampling device in accordance with the present invention;

Figures 2A to 2D show the component parts of the air sampling device shown in Figure 1;

Figure 3A shows a cross-sectional view of an air sampling device in accordance with the present invention comprising an inlet extension and wind vane;

Figure 3B shows an enlarged view of the separation gap of the air-sampling device shown in Figure 3A;

Figure 4 shows a collection vessel for use in the present invention;

Figure 5 shows plan and side views of an embodiment of the present invention comprising a virtual impactor with automated provision of collection vessels and example downstream processing steps;

Figure 6 shows a side view of an embodiment of the present invention showing the arrangement of the air intake and the wind vane (not to scale);

Figure 7 shows a side view of an embodiment of the present invention comprising a virtual impactor and traversing mechanism;

Figure 8A shows a plan view of a finger (traversing) mechanism for use in the present invention;

Figure 8B shows a cross section through the finger mechanism of Figure 8A;

Figure 8C shows a cross-section though a collection vessel heater for use in the present invention;
Figure 9 shows a side view of a carousel and round track layout for use with a virtual impactor of the present invention;

Figure 10 shows a plan view of a carousel and round track layout for use with a virtual impactor of the present invention;

Figure 11 shows the effect of separation gap on spore collection, per litre of air sampled, of Lycopodium spores (≈ 33 µm) by MVI (4 mm inlet into 6 mm collection chamber) compared to simultaneous collection in the same wind tunnel by a multivial cyclone trap. \( x = \) separation gap (mm) from fully closed, \( y = \) spores collected by MVI/by cyclone trap. The MVI trap was operated at a flow-rate of 23.7 L min\(^{-1}\) and the cyclone at 20.2 L min\(^{-1}\) in a wind tunnel with wind speed 0.85 m s\(^{-1}\). Spores were released into the airstream by a fluidized air-jet system approximately 3.5 m upwind of the samplers. Wind-tunnel cross-section was 0.65 m wide by 0.5 m high. Spore collections were quantified by microscopy using a haemocytometer slide with the MVI collection deposited passively into 250 µL water and the same volume of water was added to the cyclone collection and mixed by shaking after dry sampling; and

Figure 12 shows the effect of separation gap on spore collection, per litre of air sampled, of Lycoperdon giganticum spores (≈ 4.8 µm) by MVI (3 mm inlet into 6 mm collection chamber broadening out to 10 mm at the base of the collection chamber) compared to simultaneous collection in the same wind tunnel by a Hirst (14 x 2 mm orifice) impactor. \( x = \) separation gap (mm) from fully closed, \( y = \) spores per Litre by MVI/by Hirst trap. Both traps were operated at flow-rates of 20 L/min\(^{-1}\) in a wind tunnel with wind speed 0.85 m s\(^{-1}\). Spores were released into the airstream manually by shaking a mature giant puffball approximately 4.5 m upwind of the samplers. Wind-tunnel cross-section was 0.65 m wide by 0.5 m high. Spore collections were quantified by microscopy with the MVI collection deposited passively onto a wax-coated glass slide. The line of fit has the equation: \( y = -29.811x + 91.21 \) (Goodness of fit \( R^2 = 0.65 \)).

The present invention relates to methods, devices and apparatus for sampling air and integrated detection of airborne biological particles.
The present invention provides an efficient way to sample air for biological particles. Sampling at 20 L of air per minute, particles such as spores and pollen are deposited into a collection vial, either as a dry deposit, or into a liquid. Most other devices that sample into liquids suffer from high evaporation rates which means that the collection liquid has to be replenished or the sampling period restricted to relatively short periods. In contrast, the present invention allows sampling for up to 12 hours per day with minimal evaporation, which means spores can be deposited into culture medium to test for viable organisms, or directly into DNA extraction buffer. The present invention is compatible with a wide range of downstream diagnostic methods such as microscopy, DNA-based detection, immunological or chemical detection by biosensors, lateral flow devices, dip sticks or by optical characteristics.

The present invention provides a compact, portable, weatherproof, stand-alone unit with low power consumption. It can be powered by a 12v battery, dynamo, solar panel or mains. Long-term sampling is possible into liquid, owing to the design of the air sampling device, which minimises air transfer at the base of the collection vial.

The present invention provides high collection efficiency for particles over 2 microns aerodynamic diameter. Also provided is a post-sampling integrated system for automatic and rapid detection of specific airborne particles. Results can be obtained within two hours of the collection period for nucleic acid based methods.

The present invention is suitable for use by a non-specialist and, with a suitable power supply, the programmable integrated system can allow collection of daily samples over extended periods, for example 30 days.

Within this specification, the term "virtual impactor" means a device used to separate particles by size into two airstreams.

Within this specification, the term "about" is interpreted to mean optionally ±20%, preferably optionally ±10%, more preferably optionally ±5%.
With reference to Figure 1, there is provided an air sampling device 1 comprising a body section 2, an inlet 3 for allowing air to enter the body section, a first outlet 7 for allowing air to exit the body section towards a collection vessel 14, and a second outlet 12 for allowing air to exit the body section towards a vacuum pump (not shown).

The inlet member 3, the first outlet member 7 and second outlet 12 are configured such that when the second outlet 12 is connected to a vacuum pump (not shown), a major air flow path is created between the inlet and the second outlet 12 and a minor air flow path is created between the inlet and the first outlet 7. Airborne particles which enter the device through the inlet follow either the major air flow path or the minor air flow path according to their size. Airborne particles of greater size will follow the minor air flow path and airborne particles of lesser size will follow the major air flow path.

The inlet member 3 comprises an inlet passage 4 tapered inwardly at approximately 10.6 degrees inclusive angle. The inlet passage 4 is tapered from an inlet passage first end 5 having an inlet passage first diameter to an inlet passage second end 6 having an inlet passage second diameter. The inlet passage first diameter is greater than the inlet passage second diameter. The inlet passage first diameter 5 is between 1.3 and 1.9cm; the inlet passage second diameter 6 is between 0.2 and 0.8cm.

The first outlet member 7 comprises a first outlet passage 8 tapered from a first outlet passage first end 9 having a first outlet passage first diameter to a first outlet passage second end 10 having a first outlet passage second diameter. The first outlet passage first diameter is less than the first outlet passage second diameter. The outlet passage first diameter 9 is between 0.2 and 0.8cm; the outlet passage second diameter 10 is between 0.6 and 1.2cm.

The inlet passage 4 and the first outlet passage 8 are substantially conical and defined by the inlet member 3 and the first outlet member 7, respectively. The axis of both conical passages 4, 8 are aligned so that the inlet passage 4 and first outlet passage 8 are in alignment to form a minor air flow path between the inlet passage first end 5 and the first outlet passage second end 10. The minor air flow path is indicated in Figure 1 by arrow A. The inlet passage second end 6 and the first outlet passage first end 9 are separated by a separation gap 11, which is
measured from zero (fully closed) to a chosen gap size, which, in the example shown, is the vertical distance between the body of the inlet passage and the first outlet passage first end. The second outlet 12 is positioned for drawing air through the separation gap 11 from the minor air flow path along a major air flow path formed between the inlet first end and a vacuum pump (not shown) connected to the second outlet. The major air flow path is indicated in Figure 1 by arrow B.

In preferred embodiments, the inlet passage second diameter is about 0.4 cm and the first outlet passage first end diameter is about 0.6 cm.

In preferred embodiments, the inlet passage second diameter is about 0.3 cm, the first outlet passage first end diameter is about 0.6 cm and the first outlet passage second end diameter is about 1.0 cm.

The separation gap 11 is adjustable according to the particle size to be captured. Means 11A are provided for moving the conical inlet member 3 towards or away from the conical first outlet member 7 to adjust the separation gap 11 for optimum particle size capture.

With reference to Figures 2A to 2D there is shown in more detail the component parts of the air sampling device shown in Figure 1, together with example dimensions of the various parts described above.

With reference to Figure 3A there is shown an air sampling device 1 similar to that shown in Figure 1. The air sampling device shown in Figure 3A comprises an inlet extension 3A and wind vane 22.

As shown in greater detail in Figure 3B, the inlet passage second end 6 is located within the first outlet passage first end 9. However, the inlet passage second end 6 and first outlet passage first end 9 are still separated by a separation gap 11. The separation gap shown in Figure 3B is 0.76mm.

Also shown in Figure 3A is a cover 29, adjustable orifice 30 and rain check flange 31.
With reference to Figure 4, there is shown a detailed view of a collection vessel for use in the present invention. The collection vessel is preferably manufactured such that all surfaces are smooth and free from flashing.

The collection vessel comprises an upper section 46 and lower section 47 separated by a membrane 48.

With reference to Figures 5 and 6, there is provided an apparatus comprising an air sampling device 1 according to the present invention provided with a vacuum pump 13, a plurality of collection vessels 14, and a system of conveyors 15 and carousels 16 for transporting the collection vessels between pre-collection 14A, collection 14B and post-collection 14C positions. The apparatus further comprises a detection means 17 for analysing the content of the collection vessels. The post-collection, or down-stream, section of the apparatus includes a number of treatment/analysis stations for analysing the content of the collection device. For example, in the example shown, there is provided a heater 18, a press 19, and an LED 20. Information regarding the content of the collection vessel is transmitted to a user via a signal output device 21. Also shown is a heater timer 18A, press housing 19A, LED housing 20A, bed plate 25, traversing mechanism 26 and slider ways 27.

With reference to Figure 6, the air sampling device 1 is provided with a wind vane 22 to ensure that the inlet is aligned with the wind direction. In the example shown, the inlet has an extension 3A for alignment with the wind direction. The inlet is mounted using bearings 23 to allow the inlet to swivel in response to changing wind direction. In the example shown in Figure 6, the apparatus is provided with a metal or plastic cover 24. Also shown is a signal output control unit 28.

With reference to Figure 7, there is shown a side view of an embodiment of the present invention comprising a virtual impactor 1 and traversing mechanism 26. This embodiment includes a pot punch unit 32, rubber seal 33, card reader 34, stack 35 and stack transfer motor 36.
With reference to Figure 8A, there is shown a plan view of a finger mechanism 45 for use in the present invention. The finger mechanism 45 includes a finger bar 37, finger drive motor 38 and a plurality of fingers 39. A section through the finger mechanism is shown in Figure 8B.

With reference to Figure 8C, there is shown a cross-section though a collection vessel heater for use in the present invention.

With reference to Figure 9, there is shown a side view of a carousel 16 and round track layout for use with a virtual impactor of the present invention.

With reference to Figure 10, there is shown a plan view of a carousel and round track layout for use with a virtual impactor of the present invention. In particular, there is shown a cam plate 40, carousel top plate 16A, track centre plate 41, card stack feeder 42, card reader 34, card feeder 43, collection vessel 14 and track 44.

In one example, and in accordance with the results shown in Figure 12, the collection chamber may broaden from a 6mm collection orifice (into which the inlet jet is inserted) to 10mm at the base of the collection chamber, opening to a 10mm collection pot. This gave an improved collection efficiency of around 70% with the smallest separation gap used (0.6mm). It was not possible to decrease the separation below this distance without reducing the flow rate of the device as the area available for air to pass through would become less than the area of the 3mm diameter inlet orifice and start to restrict air flow.

Additionally, the rate of evaporation of 500microLitres of a liquid growth medium over periods of 12-24hours and a range of different weather conditions was tested. Minimal evaporation was found on cool wet days e.g. 0.5-2.5 uL lost per hour of sampling. On warm sunny conditions, this increased to between 3 to 8 uL/hour, which included over six hours between 20°C to 25°C and bright sunshine. With suitable insulation and reflective coatings, the evaporation loss even in warm sunny conditions can be minimised to negligible levels.
The device of the present invention has a number of advantages over known sampling devices, which include:

1) The device is a compact, portable, stand-alone unit that is provided complete with a suction pump-operated air sampler and a post-sampling integrated system for automatic detection of specific airborne biological particles.

2) The device can be manufactured and provided at a relatively low cost.

3) The device can be operated with a reduced power use. The device can be operated from a 12V battery, or be dynamo or solar powered, or be mains powered.

4) The compact air sampler of the present invention operating at a flow rate of 23 L/min by virtual impaction provides high sampling efficiency in the 1-30 µm range (aerodynamic diameter) with the air intake rotatable to face the prevailing wind by means of the wind vain to ensure near-isokinetic sampling.

5) The device can be automated and programmed to sample for a set period followed immediately by automatic analysis of the sample.

6) The portable nature of the device allows samples to be analysed at the device's location (regardless of whether this is an indoor or outdoor site). This allows for analysis immediately after sampling (within 2 hours of the end of the collection period for nucleic acid based methods).

7) All sampling and result(s) are produced automatically.

8) Results from the device can be output means such as a GSM text etc.

9) Sample times are user defined and user programmable. This means that the device is easy set up and no scientific knowledge is required to operate the device (farmer friendly).
10) Each device can work independently or can be set up to create a network.

11) The way spores are passively deposited into liquid growth media makes the device excellent for monitoring for viable organisms - most other devices either sample into liquid that needs topping-up due to evaporation, or can only sample for a short period, or those sampling onto solid agar can give a misleadingly reduced level of contamination if some viable spores are actually killed on impaction.

The present invention provides integrated detection of airborne biological particles comprising a volumetric air sampler operating by virtual impaction followed by an automated processing of the collected sample leading to an electronic signal indicating the presence of the chosen agent.

The method of detection of the chosen biological agent may be by chemical, immunological or nucleic-acid-based detection. The detection method may be of a specific sequence of DNA and recorded by measurement of colour or fluorescence, which is translated into an electronic signal.

The detection method may be enhanced by an intermediate, automated processing step to release DNA or other cell contents that then form the basis of detection. The intermediate step and/or detection step may be enhanced by heating of the sample and may use a cell lysis or DNA extraction product. In preferred embodiments, the detection method may involve recombination proteins, recombinase polymerase amplification or Loop Mediated Amplification (LAMP, Eiken Chemical), or polymerase chain reaction (PCR, qPCR), mediated by primers to target specific sequences of DNA or RNA. The detection may be enhanced further by use of a probe containing a fluorophore and quencher system.

The detection method may involve antibody-based binding of specific protein or other molecules, leading to detection by immunosensor (electrochemical change) or colour/fluorescence detection of target-bound antibodies with a second dye-labelled antibody.
The detection method may involve quantification of light (fluorescence following an excitatory light pulse, or light absorbance), or an electrochemical detection method.

The detection method may be either by direct chemical detection of a metabolite produced by the target biological particle or by indirect detection of the product following an enzymatic reaction, both cases leading to translation of a change in electrical conductance, resistance or capacitance that can be measured.

The sample may be collected into a chamber pre-loaded with reagents to facilitate the release of cell contents or could be collected as a dry sample with a sufficient dose of cell-lysis reagents added automatically after sampling. The resulting liquid suspension of cell contents may be transferred to a second chamber, preloaded with reagents, or with reagents supplied from a reservoir as necessary for the detection of target molecules.

Alternatively, the resulting liquid suspension of cell contents may be purified to remove inhibitors and then the remaining semi-purified extract (e.g. nucleic acid) exposed to reagents necessary for the detection of target molecules.

The sensor may be adapted to detect multiple agents either by separation of the cell extract into sub-samples that can undergo detection in separate containers or by dosing onto a single surface, preloaded with spots of different known specific primers and associated reagents for nucleic acid-based detection, with each spot separated by a printed grid of hydrophobic substance to facilitate separation of the cell extract into sub-samples.

The result of the detection assay may be translated into an electronic signal either by measurement of light at a specific wavelength following excitation of the assay(s) by a light source emitting at a shorter wavelength, or by absorbance of light transmitted through the assay(s) and normalised by transmission of light at a wavelength that is unaffected by the assay. The resulting electronic output may be transmitted wirelessly by means such as a GSM text or by a wire-based system within 2 hours of the end of sample collection.
The present invention can be used to detect both fungal plant pathogens and other airborne biological particles, for example pathogens of human or animals, food spoilage agents, allergens, GM pollen, GMOs, fungicide resistant variants of a fungus, antibiotic resistant variants of a bacterium, and so on. In one example, the invention can be used to detect pathogenic bacteria. However, in general, the invention can be used to detect pathogens in a variety of application areas, including for example: healthcare (e.g. *Aspergillus niger, Aspergillus fumigatus, Staphylococcus aureus, Saccharomyces cerevisiae*), animal health (e.g. *Aspergillus niger*), environmental monitoring (e.g. *Sclerotinia sclerotiorum, Botrytis cinerea, Puccinia spp, Fomitopsis palustris*), food spoilage (e.g. *Clostridium botulinum*), post-harvest grain storage (e.g. *Burkholderia glumae*), materials protection (e.g. *Fomitopsis palustris*) and bio-security (e.g. *Bacillus anthracis*).

It should be understood that various changes and modifications to the presently preferred embodiments described herein will be apparent to those skilled in the art. Such changes and modifications can be made without departing from the spirit and scope of the present invention and without diminishing its attendant advantages. It is therefore intended that such changes and modifications are covered by the appended claims.
CLAIRMS

1. An air sampling device which comprises:-
   
   (v) a body section;
   
   (vi) an inlet for allowing air to enter the body section;
   
   (vii) a first outlet for allowing air to exit the body section towards a collection vessel; and
   
   (viii) a second outlet for allowing air to exit the body section towards a vacuum pump,

   the inlet, first outlet and second outlet being configured such that when the second outlet is connected to a vacuum pump, a major air flow path is created between the inlet and the second outlet and a minor air flow path is created between the inlet and the first outlet, and airborne particles which enter the device through the inlet follow either the major air flow path or the minor air flow path according to their size,

   the inlet comprising an inlet passage tapered from an inlet passage first end having an inlet passage first diameter to an inlet passage second end having an inlet passage second diameter, the inlet passage first diameter being greater than the inlet passage second diameter,

   the first outlet comprising a first outlet passage tapered from a first outlet passage first end having a first outlet passage first diameter to a first outlet passage second end having a first outlet passage second diameter, the first outlet passage first diameter being less than the first outlet passage second diameter,

   the passages of the inlet and first outlet being substantially in alignment to form a minor air flow path between the inlet first end and the first outlet second end,

   the inlet second end and first outlet first end being separated by a separation gap,

   the second outlet being positioned for drawing air through the separation gap from the minor air flow path along a major air flow path formed between the inlet first end and a vacuum pump connected to the second outlet,

   wherein the inlet passage first diameter is between about 1.3 cm and about 1.9 cm, the inlet passage second diameter is between about 0.2 cm and about 0.8 cm, the first outlet passage first diameter is between about 0.2 cm and about 0.8 cm, the first outlet passage second diameter is between about 0.6 cm and about 1.2 cm.
2. An air sampling device according to claim 1, wherein the inlet passage second diameter is less than or equal to the first outlet passage first diameter.

3. An air sampling device according to claim 1 or 2, wherein the inlet passage is between about 3.0 cm and about 3.5 cm in length.

4. An air sampling device according to any preceding claim, wherein the first outlet passage is between about 1.8 cm and about 2.3 cm in length.

5. An air sampling device according to any preceding claim, wherein the separation gap is adjustable.

6. An air sampling device according to any preceding claim, wherein the separation gap is less than about 2mm.

7. An air sampling device according to any preceding claim, wherein the inlet passage second end is located within the first outlet passage first end.

8. An air sampling device according to any preceding claim, wherein the major airflow path is non-linear and the minor airflow path is substantially linear, such that heavier airborne particles will follow the minor airflow path and lighter airborne particles will follow the major airflow path.

9. An air sampling device according to any preceding claim, wherein the inlet and first outlet are at opposing ends of the air sampling device.

10. An air sampling device according to any preceding claim, wherein the second outlet is positioned between the inlet and the first outlet, preferably extending from a side wall of the air sampling device.

11. An air sampling device according to any preceding claim, which comprises a means for directing the inlet into the path of a wind.
12. An air sampling device according to claim 11, which comprises one or more baffle plates, for example a wind vane.

13. An air sampling device according to claim 11 or 12, which comprises a rotatable means for directing the inlet into the path of the wind.

14. An air sampling device according to any preceding claim, which comprises a vacuum pump connected to the second outlet.

15. An air sampling device according to any preceding claim, configured to operate at an airflow rate of between about 15 L/min and about 30 L/min.

16. An air sampling device according to any preceding claim having a width of less than about 8 cm and a depth of less than about 8 cm, optionally having a height of less than about 8 cm.

17. An apparatus for detecting airborne biological particles, the apparatus comprising:
   (iv) a virtual impactor,
   (v) one or more collection vessels for collecting biological particles which pass through the virtual impactor,
   (vi) one or more detection means for detecting biological particles within the one or more collection vessels.

18. An apparatus according to claim 17, wherein the virtual impactor is an air sampling device according to any of claims 1 to 16.

19. An apparatus according to claim 17 or 18, wherein the one or more detection means permit detection of biological particles by chemical, immunological or nucleic acid based detection.
20. An apparatus according to any of claims 17 to 19, further comprising an output means for transmitting an output signal, for example a detection signal, from the detection means to a user.

21. An apparatus according to claim 20, wherein the output means transmits a detection signal to a user wirelessly, for example by GSM text.

22. An apparatus according to any of claims 17 to 21, wherein the virtual impactor is rotatable for positioning an inlet of the virtual impactor into alignment with wind direction.

23. An apparatus according to any of claims 17 to 22, wherein the one or more collection vessels contain reagents for use by the one or more detection means.

24. An apparatus according to any of claims 17 to 23, comprising means for adding reagents to the one or more collection vessels, for example after collection of biological particles from the virtual impactor.

25. An apparatus according to any of claims 17 to 24, comprising collection vessel conveying means for moving the one or more collection vessels from a pre-collection position to a collecting position, and from a collecting position to a post-collecting position.

26. An apparatus according to any of claims 17 to 25, wherein the apparatus is controlled by a computer.

27. An apparatus according to any of claims 17 to 26, wherein the apparatus is configured to collect air samples for set time periods.

28. An automated method for detecting airborne biological particles, the method comprising use of an air sampling device according to any of claims 1 to 16 or apparatus according to any of claims 17 to 27.
29. A method according to claim 28, comprising obtaining a sample of air, analysing the sample of air for the presence of biological particles, outputting a signal corresponding to the presence or absence of biological particles to a user, preferably via wireless communication, for example via GSM text or email.

30. A method according to claim 29, wherein the signal provides information regarding the amount and identity of the biological particles.

31. A method according to any of claims 28 to 30, wherein the method comprises extracting DNA and/or a metabolite from biological particles in the sample of air, detecting DNA of a specific sequence and/or a target metabolite, and outputting a signal corresponding to the presence or absence of the DNA or metabolite.
Fig 1

45.5mm O/D
x 39.5mm I/D
APPROX DIMN. FOR ø3mm 'O' RING

ø1.793 ± 0.001" x 39.5mm I/D

45.5mm O/D

ø1.996 ± 0.001"

5/8" x 26

ø3.7mm

CAP
(Fig 2A)
(Fig 2C)

(Fig 2D)