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(54) APPARATUS AND METHOD FOR AUTOMATED MONITORING OF AIRBORNE BACTERIAL SPORES
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ABSTRACT
An apparatus and method for automated monitoring of airborne bacterial spores. The apparatus is provided with an air sampler, a surface for capturing airborne spores, a thermal lysis unit to release DPA from bacterial spores, a source of lanthanide ions, and a spectrometer for excitation and detection of the characteristic fluorescence of the aromatic molecules in bacterial spores complexed with lanthanide ions. In accordance with the method: computer-programmed steps allow for automation of the apparatus for the monitoring of airborne bacterial spores.

23 Claims, 3 Drawing Sheets
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FIGURE 3

S1 Instrument Initialization
1 min

S2 Define Parameters
e.g. threshold

S3 Air Sampling
15 min

S4 Thermal Lysis Unit
15 sec, 200°C

S5 Lanthanide Solution Addition (TbCl₃)

S6 Spectrophotometer Measurement (UV light, fluorescence detection)

S7 Data Storage

S8 Above Threshold?
Yes Alarm On
No

S9 Sampling Time End?
Yes Sampling Completed
No
According to a second aspect of the present disclosure, a method for the apparatus as disclosed above is provided, the method comprising the steps of: (a) operating the air sampler to deposit sampled air onto the surface; (b) moving the surface from the air sampler to a position proximal to the thermal lysis unit; (c) positioning the thermal lysis unit onto the surface, thereby allowing for thermal lysis of any spores on the surface, thus releasing DPA from any lysed spores; (d) positioning the thermal lysis unit off of the surface to a position proximal to the source of lanthanide ions; (e) operating the source of lanthanide ions to provide lanthanide ions to the surface, thereby putting the lanthanide ions in contact with any DPA on the surface, resulting in said DPA-lanthanide complex; (f) moving the surface to a position away from the source of lanthanide ions to a position proximal to the spectrometer comprising a UV source; (g) operating the spectrometer to expose the surface to the UV source, thereby exciting said DPA-lanthanide complex and producing fluorescence; (h) operating the spectrometer to detect the fluorescence resulting from the excited DPA-lanthanide complex of step (g); and (i) quantifying the fluorescence intensity and/or imaging the fluorescence of step (h).

According to a third aspect of the present disclosure, a method for the apparatus as disclosed above is provided, the method comprising the steps of: (a) operating the air sampler to deposit sampled air onto the surface; (b) moving the surface from the air sampler to a position proximal to a source of lanthanide ions; (c) operating the source of lanthanide ions to provide lanthanide ions to the surface; (d) moving the surface to a position away from the source of lanthanide ions to a position proximal to the thermal lysis unit; (e) moving the thermal lysis unit onto the surface, thereby allowing for thermal lysis of any spores on the surface, thus releasing DPA from any lysed spores, and putting DPA in contact with the lanthanide ions provided to the surface in step (c) to form said DPA-lanthanide complex; (f) moving the thermal lysis unit off of the surface to a position proximal to a spectrometer comprising a UV source; (g) operating the spectrometer to expose the surface to the UV source, thereby exciting said DPA-lanthanide complex and producing fluorescence; (h) operating the spectrometer to detect the fluorescence resulting from the excited DPA-lanthanide complex of step (g); and (i) quantifying the fluorescence intensity and/or imaging the fluorescence of step (h).

One advantage of a bacterial monitor of the present disclosure, is that the chemical marker used for detection—DPA—is essential to the viability of the bacterial spore yet is present whether the spores are alive or dead. Furthermore, since approximately 10^9 molecules of DPA are present in each bacterial spore as compared to 1 copy of DNA, expensive sample amplification schemes are not required, which significantly lowers the complexity and cost of the monitoring apparatus. Furthermore, DPA binding to terbium exhibits a million-fold greater binding constant than amino acids or nucleic acids.

Another advantage of the present disclosure is that it is not susceptible to false positives due to natural fluctuations in bacterial spore concentrations because these fluctuations occur in a concentration regime many orders of magnitude lower (natural concentrations range from 0.01-0.5 bacterial spores per liter of air) than those, for example, present during an anthrax attack (Pastuszka, J. S. et al., 2000, Atmospheric Environment, 34, 3833-3842).

A further advantage of the present disclosure is that unlike other fluorescence detection methods, the DPA triggered lanthanide luminescence method for bacterial spore detection is not mired by varying background fluorescence from interfe-
manner to control the apparatus (FIGS. 2, 3).

providing terbium or europium ions to the DPA released from

rescence lifetimes from impurities are generally on the order

UV source for excitation of the DPA-lanthanide complex

soures marketed for automated monitoring of bacterial spores in accor-

ion (terbium, europium) luminescence (Hindle and Hall,

dance with a first embodiment of the present disclosure.

A brief description of the drawings

FIG. 1 shows an air sampler with quartz fiber tape on spools

FIG. 2 shows a computer-operated, automated apparatus

FIG. 3 shows a flow chart of steps of a computer program

Detailed description

Rapid detection of bacterial spores of the present disclosure

Rapid detection of bacterial spores of the present disclosure

An apparatus according to the present disclosure herein, enables

A source of lanthanide ions (4) (terbium or europium)

A thermal lysis unit (3) can be of any type so long as its

A thermal lysis unit (3) can be of any type so long as its

An apparatus according to the present disclosure herein, enables

An apparatus according to the present disclosure herein, enables

A computer program as outlined

A computer program as outlined

In one embodiment of the present disclosure, an automated

In one embodiment of the present disclosure, an automated

In a second embodiment of the present disclosure, an apparatus

In a second embodiment of the present disclosure, an apparatus
In another embodiment, the apparatus of the present disclosure is computer-operated (FIGS. 2, 3). A computer-operated/automated apparatus comprises a computer that is programmed to control each component of the apparatus. This automated apparatus does not require outside intervention apart from establishing the program parameters and other computer input functions. This automated apparatus further comprises a computer program as outlined in FIG. 3.

For example, in an automated detector of the present disclosure, the air sampler (1) is controlled by a computer program and motorized nozzle (8) to deposit the sampled air onto a surface (2) connectable to the air sampler (S3, FIG. 3). The air sampler is further controlled with respect to the length of air sampling time. The surface for deposit of the air sample is defined as anything to which spores in the air will adhere. The surface of an automated apparatus is positioned by way of computer and motorized spools (6) that are programmed to move from a position proximal to the air sampler to a position proximal to a thermal lysis unit (3).

Movement of the thermal lysis unit is also controlled by way of a computer and motorized arm (9) allowing for the thermal lysis unit to be moved from a position proximal to the surface to a position in contact with the surface, thereby allowing for thermal lysis of any spores on the surface (S4, FIG. 3).

The temperature of the lysis unit and the length of contact with the surface are parameters of the computer program. After the lysis unit provides heat to the surface, the lysis unit is computer and motor controlled to move from the surface. Once this has occurred, by way of the computer program and motorized spools, the surface moves to a position proximal to a lanthanide source (4).

The lanthanide source contains, preferably terbium ions, but could also contain europium ions. Through the programmed commands of the computer, the lanthanide source provides the lanthanide ions (SS, FIG. 3) to the surface by way of a dropper (10). In this way, lanthanide ions are in contact with any lysed spores on the surface, which as a result of lysis, release DPA onto the surface, and allow for the formation of DPA-lanthanide complexes on the surface. After the lanthanide ions are provided to the surface, the computer program and motorized spools move the surface to a position proximal to a spectrometer (5).

The spectrometer is programmed to administer UV light via a Xenon flash lamp (laser or UV LED can also be used). This UV light exposure of the DPA-lanthanide complex excites the complex, thereby creating a fluorescence which is detected using the lifetime-gated fluorescence spectrometer, and captured as a computer image (S6, S7, FIG. 3). Such an automated apparatus as disclosed is shown in FIG. 2.

The computer is further programmed to sound an alarm if the sampled air results in a fluorescence that exceeds or meets a programmed threshold fluorescence (S2, S8, FIG. 3). A flow chart detailing the computerized functions for operating the apparatus is shown in FIG. 3.

In an additional embodiment, the apparatus of the present disclosure is programmed to continually sample air, thus the steps from air sampling to analyzing the fluorescence as disclosed can be programmed to continually repeat, or repeat for a given period of time.

In an alternative embodiment, after the surface is moved from the air sampler, the surface can be moved to a position proximal to a lanthanide source for lanthanide ions, and then subsequently moved to a position proximal to the thermal lysis unit. In other words, the thermal lysis step (S4) and the step of providing lanthanide ions (SS) can be transposed.

EXAMPLE 1
Air Sampler

For capture of aerosolized bacterial spores according to the present disclosure, a Model 7001 air sampler (Universal Detection Technology, Beverly Hills, Calif.) is shown in FIG. 1. This Model 7001 air sampler has a high transfer efficiency (>95%) for microbe-containing aerosols, and it uses a glass fiber filter tape. The air sampler shown in FIG. 2 was customized for the automated apparatus as shown. The top half of the assembly leads to the air inlet, and the bottom connects to the vacuum pump. A motorized nozzle (8) makes an airtight connection during air sampling, and is retracted after sampling to allow the tape to move forward. Similar air samplers can be used.

EXAMPLE 2
Surface for Capture of Spores

The surface (2) for capturing spores from the air sampled and taken in by the air sampler was a quartz glass fiber filter tape (Whatman) custom made for the Model 7001 air sampler (FIG. 1). Quartz fiber tape collects micron-sized particles as air is pulled through the filter. The same quartz fiber tape is used in the automated apparatus shown in FIG. 2.

EXAMPLE 3
Implement for Thermal Lysis of Spores

The thermal lysis unit (3) in FIG. 2 (consists of a soldering iron with a flat tip mounted on a motorized stage. To lyse (pop) spores the thermal lysis unit makes contact with the surface at high temps (~200° C.).

EXAMPLE 4
Source of Lanthanide Ions

The source of lanthanide ions (4) used in the present disclosure consisted of a dropper (10) providing 25 μl from a reservoir of 10-μM TbCl₃ (terbium chloride hexahydrate, 99.999%, Aldrich, Milwaukee, Wis.) (FIG. 2). In FIG. 2, the source of lanthanide ions is connected to the surface of the apparatus, adjacent to the lanthanide ion dropper (10).

EXAMPLE 5
Lifetime-Gated Fluorescence Spectrometer

The lifetime-gated fluorescence spectrometer (5) used in the present disclosure is a miniature spectrometer with fiber-optic probe (Ocean Optics, USB2000-FL-2048-element fluorescence spectrometer and PX-2—Pulsed Xenon Flash Lamp, Dunedin Fla.). The fiber optic probe contains both fibers for excitation and detection. For background, the luminescence spectrum of 25 μl of 10-μM TbCl₃ on the fiber tape was subtracted from all luminescence spectra (FIG. 2).

EXAMPLE 6
Computer for Operation of Detector

The computer for operating the automated detector of the present disclosure was programmed according to the outline
in FIG. 3. The computer used WINDOWS (Microsoft) and LabVIEW (National Instruments Corp.) software programs.

While several illustrative embodiments have been shown and described in the above description, numerous variations and alternative embodiments will occur to those skilled in the art. Such variations and alternative embodiments are contemplated, and can be made without departing from the scope of the invention as defined in the appended claims.

The invention claimed is:

1. An apparatus for the detection of airborne bacterial spores, wherein the apparatus comprises:
   an air sampler comprising an intake; a surface connectable to the air sampler, the surface suitable for spore adhesion and on-surface spore detection; a thermal lysis unit for lysing the spores on the surface resulting in release of dipicolinic acid (DPA); a source of lanthanide ions for providing lanthanide ions to the released DPA on the surface to form a DPA-lanthanide complex; and a lifetime-gated fluorescence spectrometer having a UV source for on-surface excitation of the DPA-lanthanide complex resulting in fluorescence and subsequent detection of the fluorescence from the surface, wherein the surface is connectable to the air sampler through a relative movement between the surface and the air sampler.

2. The apparatus of claim 1, wherein the air sampler is moveable between a first condition where the air sampler is proximal to the surface, and a second condition where the air sampler forms an airtight seal with the surface.

3. The apparatus of claim 1, further comprising at least two spools, the surface connectable to the intake of the air sampler being located on said spools.

4. The apparatus of claim 1, wherein the surface connectable to the air sampler is selected from the group consisting of quartz fiber tape, electrostatically charged tape and polymer tape.

5. The apparatus of claim 1, wherein the thermal lysis unit is selected from the group consisting of a cautzerizer and a soldering iron.

6. The apparatus of claim 1, wherein the source of lanthanide ions comprises terbium ions.

7. The apparatus of claim 1 wherein the source of lanthanide ions comprises europium ions.

8. The apparatus of claim 1, wherein the source of lanthanide ions comprises a reservoir comprising a dropper for providing lanthanide ions to the surface.

9. The apparatus of claim 8 wherein the dropper administers 25 µl drops of 10 µM terbium chloride.

10. The apparatus of claim 1, wherein the UV source is selected from the group consisting of a Xenon flash lamp, a laser, and a UV LED.

11. A method for the operation of the apparatus of claim 1, the method comprising steps of: (a) operating the air sampler to deposit sampled air onto the surface; (b) moving the surface from the air sampler to a position proximal to the thermal lysis unit; (c) positioning the thermal lysis unit onto the surface, thereby allowing for thermal lysis of any spores on the surface, thus releasing DPA from any lysed spores; (d) positioning the thermal lysis unit off of the surface to a position proximal to the spectrometer comprising a UV source; (e) moving the thermal lysis unit onto the surface, thereby allowing for thermal lysis of any spores on the surface, thus releasing DPA from any lysed spores, and putting DPA in contact with the released DPA on the surface to form a DPA-lanthanide complex; (f) moving the thermal lysis unit from the air sampler to a position proximal to a source of lanthanide ions; (g) operating the spectrometer to detect the fluorescence resulting from the excited DPA-lanthanide complex of step (g); and (i) quantifying the fluorescence intensity and/or imaging the fluorescence of step (h).

12. A method for the operation of the apparatus of claim 1, the method comprising steps of: (a) operating the air sampler to deposit sampled air onto the surface; (b) moving the surface from the air sampler to a position proximal to a source of lanthanide ions; (c) operating the source of lanthanide ions to provide lanthanide ions to the surface; (d) moving the surface to a position away from the source of lanthanide ions to a position proximal to the thermal lysis unit; (e) moving the thermal lysis unit onto the surface, thereby allowing for thermal lysis of any spores on the surface, thus releasing DPA from any lysed spores, and putting DPA in contact with the lanthanide ions provided to the surface in step (c) to form said DPA-lanthanide complex; (f) moving the thermal lysis unit off of the surface to a position proximal to the spectrometer comprising a UV source; (g) operating the spectrometer to expose the surface to the UV source, thereby exciting said DPA-lanthanide complex and producing fluorescence; (h) operating the spectrometer to detect the fluorescence resulting from the excited DPA-lanthanide complex of step (g); and (i) quantifying the fluorescence intensity and/or imaging the fluorescence of step (h).

18. The apparatus of claim 17, wherein the apparatus is adapted for multiple detections each detection occurring on a different portion of the surface, through a relative movement between the surface and the air sampler.

19. The apparatus of claim 17, further comprising an arrangement for relative movement between the surface and the air sampler to adapt the apparatus to multiple detections, each detection occurring on a different portion of the surface.
20. An apparatus for the detection of airborne bacterial spores, wherein the apparatus comprises:
   an air sampler comprising an intake;
   a surface connectable to the air sampler, the surface suitable for spore adhesion and on-surface spore detection;
   a thermal lysis unit for lysing the spores on the surface resulting in release of dipicolinic acid (DPA);
   a source of lanthanide ions for providing lanthanide ions to the released DPA on the surface to form a DPA-lanthanide complex; and
   a lifetime-gated fluorescence spectrometer having a UV source for on-surface excitation of the DPA-lanthanide complex resulting in fluorescence and subsequent detection of the fluorescence from the surface, wherein the surface is connectable to the source of lanthanide ions or the lifetime-gated fluorescence spectrometer through a relative movement of the surface with respect to the source of lanthanide ions or the lifetime-gated fluorescence spectrometer.

21. The apparatus of claim 20, wherein the apparatus is adapted for multiple detections each detection occurring on a different portion of the surface, through a relative movement between the surface and source of lanthanide ions or the lifetime-gated fluorescence spectrometer.

22. The apparatus of claim 20, further comprising an arrangement for relative movement between the surface and the source of lanthanide ions or the lifetime-gated fluorescence spectrometer to adapt the apparatus to multiple detections, each detection occurring on a different portion of the surface.

23. A method for the operation of the apparatus of claim 13, the method comprising steps of: (a) operating the air sampler to deposit sampled air onto the surface; (b) moving the surface from the air sampler to a position proximal to the thermal lysis unit; (c) positioning the thermal lysis unit onto the surface, thereby allowing for thermal lysis of any spores on the surface, thus releasing DPA from any lysed spores; (d) positioning the thermal lysis unit off of the surface to a position proximal to the source of lanthanide ions; (e) operating the source of lanthanide ions to provide lanthanide ions to the surface, thereby putting the lanthanide ions in contact with any DPA on the surface, resulting in said DPA-lanthanide complex; (f) moving the surface to a position away from the source of lanthanide ions to a position proximal to the spectrometer comprising a UV source; (g) operating the spectrometer to expose the surface to the UV source, thereby exciting said DPA-lanthanide complex and producing fluorescence; (h) operating the spectrometer to detect the fluorescence resulting from the excited DPA-lanthanide complex of step (g); and (i) quantifying the fluorescence intensity and/or imaging the fluorescence of step (h); (j) comparing the fluorescence of step (h) with a programmed fluorescence threshold; (k) sounding the alarm if the fluorescence of step (h) exceeds and/or meets the programmed fluorescence threshold.

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