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## Capnography Filter Device

### Technical Field

5 The present invention relates, in general terms, to a capnography filter device.

### Background

10 Capnography refers to a continuous noninvasive measurement of the partial pressure of carbon dioxide (CO<sub>2</sub>) in exhaled breath expressed as the CO<sub>2</sub> concentration over time. It is a waveform that shows evidence of breathing. It is used quite extensively in anesthesia, critical care settings and the operating theatre.

15 Capnography provides information of breath-to-breath ventilation, and a graph can predict hyperventilation from inadequate analgesia or sedation, as well as hypoventilation from over-sedation or other potentially life-threatening causes. The diagnosis of respiratory difficulty from various obstructive mechanisms can be deduced from capnography. Capnography has been known to assist in predicting and averting impending respiratory arrest situations in hospitalized, critically ill patients who show  
20 significantly elevated CO<sub>2</sub> levels (hypercapnia).

Moderate sedation is the use of sedative medications (anxiolytics—midazolam, diazepam; opioids—morphine, fentanyl; anesthetic solutions—propofol, etomidate, ketamine) to provide patients with comfort, relaxation, amnesia, and analgesia in order  
25 to perform certain clinical procedures in various settings like cardiac catheterization laboratories, endoscopy suites, radiology, emergency rooms, ambulatory surgical centers, and doctors' offices.

Anesthesia personnel usually provide moderate sedation for procedures and safe  
30 techniques are encouraged with capnography being of paramount importance alongside other standard monitoring techniques (pulse oximetry, blood pressure, EKG). Capnography during moderate and deep sedation was added by the American Society of Anesthesiologists as part of the Standards for Basic Anesthetic Monitoring, effective July 1, 2011. Anesthesia personnel have recognized capnography as the appropriate  
35 measure of adequacy of ventilation during non-intubated procedures requiring moderate

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or deep sedation.

Meta-analysis of 13 RCTs published between 2006 and 2016 showed a reduction in respiratory compromise (from respiratory insufficiency to failure) during procedural sedation and analgesia (PSA) with the inclusion of capnography monitoring. Two closed claim reviews both found that inadequate oxygenation/ventilation was the most frequent event leading to a claim related to PSA outside the operating room. The potential cost burden is demonstrated by the median cost of a claim settled being US\$300,000.

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Increasingly, capnography is being used in moderate and deep sedation cases in hospitals all over the world. The monitoring of ventilation using capnography is vital to titrating sedatives for which different patients have variable levels of sensitivity. It will provide early warning signs of adverse respiratory events as well as assist in better patient care and outcomes.

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However, there may be potential issues such as cross-contamination as the tubings used are transferred between patients. Accordingly, many health care providers have chosen to provide regional anesthesia without sedation during the pandemic for fear of inadequate infection control with contaminated capnography monitors. This has slowed down the progress of capnography as being a standard procedure. This may also compromised the comfort of some of the non-sedated anxious patients during surgeries.

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It would be desirable to overcome or ameliorate at least one of the above-described problems.

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### Summary

The present disclosure concerns a capnography filter device, comprising:

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- a) an elongated housing having a first end and a second end, the first and second ends each independently comprising a protrusion;
  - b) at least one filter housed within the housing;
- wherein the filter is characterized by a pore size of about 0.2  $\mu\text{m}$  to about 0.5  $\mu\text{m}$ .

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In some embodiments, the at least one filter is in contact with an inner side of the

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housing at the first end or second end.

In some embodiments, the housing is characterized by an inner diameter configured to be substantially similar to an inner diameter of an attachable tubing when in use.

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In some embodiments, the housing is separable in order for an interior space of the housing to be accessed.

In some embodiments, the housing further comprises connecting means.

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In some embodiments, the capnography filter device further comprises an insert with a through hole.

In some embodiments, the insert is configured to frictionally fit inside an interior space of the housing and configured to engage the at least one filter.

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In some embodiments, the insert is a Teflon insert.

In some embodiments, the housing is characterized by an outer diameter of about 5 mm to about 15 mm.

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In some embodiments, the housing is characterized by an outer diameter of about 9 mm.

In some embodiments, the housing is characterized by an inner diameter of about 2.5 mm to about 4.5 mm.

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In some embodiments, the housing is characterized by an inner diameter of about 4 mm.

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In some embodiments, the housing is characterized by a length of about 15 mm to 30 mm.

In some embodiments, the housing is characterized by a length of about 22 mm.

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In some embodiments, the housing is characterized by an inner capacity of about 60 mm<sup>3</sup> to about 1000 mm<sup>3</sup>.

In some embodiments, the at least one filter is two filters.

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In some embodiments, the two filters are spaced apart.

In some embodiments, the two filters are each independently in contact with respective inner sides of the housing at the first end and second end.

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In some embodiments, the at least one filter is a membrane filter, HEPA filter or N95 filter.

15 In some embodiments, the at least one filter comprises an anti-microbial composition, an anti-viral composition, or a combination thereof.

In some embodiments, the anti-microbial and/or anti-viral composition comprises:

a) a cashew testa extract; and

20 b) iron-iron oxide core-shell particles, the core is an elemental iron core or an iron alloy core, and the shell is an iron oxide shell;

wherein the cashew testa extract comprises a component selected from amino acid, protein, carbohydrate, phenolic compounds or a combination thereof; and wherein the iron-iron oxide core-shell particles are at least partially passivated by a component of the cashew testa extract.

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In some embodiments, the phenolic compounds are selected from tannins, catechin, epicatechin, epigallocatechin, p-coumaric, gallic acid, or a combination thereof.

30 In some embodiments, the iron-iron oxide core-shell particles is characterized by a particle size of about 1 μm to about 100 μm.

In some embodiments, the iron-iron oxide composition relative to the at least one filter is about 0.1% w/w to about 5% w/w.

35 In some embodiments, the shell has a thickness of about 50 nm to about 400 nm.

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In some embodiments, the shell further comprises at least a component of the cashew testa extract.

- 5 In some embodiments, the anti-microbial composition further comprises an excipient selected from a surfactant, a stabilizer, a polymer, drying agent or a combination thereof.

- 10 In some embodiments, the capnography filter device is characterized by a particulate filtration efficiency at 0.1  $\mu\text{m}$  of more than about 90%.

In some embodiments, the capnography filter device is characterized by an anti-bacterial activity of at least 2 log reduction.

- 15 In some embodiments, the capnography filter device is characterized by an anti-viral activity of at least 2 log reduction.

#### **Brief description of the drawings**

- 20 Embodiments of the present invention will now be described, by way of non-limiting example, with reference to the drawings in which:

**Figure 1** shows a schematic of the membrane filter device.

**Figure 2** shows a photograph of the membrane filter device.

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#### **Detailed description**

- The present disclosure is predicated on the understanding that capnography may improve a patient's safety outside the operating room. In this regard, capnography may be an appropriate measure of adequacy of ventilation during nonintubated procedures which requires moderate or deep sedation. Accordingly, to improve acceptance of capnography as a mainstream procedure in the healthcare setting, further improvements may be needed.
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- 35 Further, during the COVID-19 pandemic, the presence of an effective viral filter may

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provide assurance for anaesthesiologists and/or sedationists to continue to monitor breathing pattern of sedated patients via presence of exhaled carbon dioxide waveform without fear of cross contamination and environmental pollution.

- 5 The present disclosure concerns a capnography filter device that allows for attachment to a side stream monitoring line of a capnography system for reducing cross contamination between patients. The capnography filter device is for filtering an exhaled breath of a patient. The capnography filter device may have an anti-microbial filter. The device may prevent potential contamination of bacteria and viruses from the patient
- 10 to the capnography system via the membrane filter. The device may be a single use device. This may allow health care professionals to provide the appropriate anesthesia care with sedation to patients with adequate infection control during a pandemic.

Accordingly, the present disclosure concerns a capnography filter device, comprising:

- 15 a) an elongated housing having a first end and a second end, the first and second ends each independently comprises a protrusion;
- b) at least one filter housed within the housing and adjacent to the first end; wherein the filter is characterized by a pore size of about 0.2  $\mu\text{m}$  to about 0.5  $\mu\text{m}$ .

- 20 The capnography filter device 10 is shown in Figure 1. The filter device 10 comprises a housing 102. The housing 102 has a first end 104a, and a second end 104b. The first end 104a comprises a protruding member or protrusion 106a (first protrusion). The second end 104b comprises a protruding member or protrusion 106b (second protrusion). The protrusions 106a and 106b each comprises an outlet, such that an
- 25 interior space 114 within the housing 102 is fluidly communicated with an exterior environment. The filter device comprises at least one filter 108a or 108b within the housing 102. Figure 1 shows a filter device with two filters 108a and 108b. The at least one membrane filter 108a or 108b is positioned adjacent to either the first end 104a or the second end 104b. The at least one membrane filter 108a or 108b may be in contact
- 30 with an inner side of the housing 102 at the first end 104a or second end 104b, or may be flushed against an inner side of the first end 104a or second end 104b. This reduces entry of microbes into the interior space 114 of the housing 102. The at least one membrane filter 108a or 108b is positioned such that airflow from the first end 104a to the second end 104b and vis versa flows through the at least one membrane filter 108a
- 35 or 108b. This allows the CO<sub>2</sub> flow from the patient to travel through the membrane

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filter, which may filter out bacteria and viruses such as *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 15442, *Enterococcus faecium* ATCC 6057, *Salmonella typhimurium* ATCC 13311 and SARS Cov-2.

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The housing 102 may have an elongated morphology. It is believed that this slows down the transfer of microbes between the two ends.

10 The protrusions 106a and 106b are each adapted to frictionally fit into a tubing, such that no loss of exhaled air may occur from the interface of the connection. In order to ensure a tight fit, the protrusions 106a and 106b may each be tapered towards its end thereof. The protrusions 106a and 106b may additionally comprise grooves. The grooves may assist to frictionally engage a tubing. Alternatively, an adaptor may be used to facilitate the fitting of the protrusions 106a and 106b to the capnography system.

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The protrusions 106a and 106b may be similarly sized. Alternatively, one protrusion may be smaller or larger than the other.

20 The housing 102 may have an inner diameter which is sized to substantially similar to an inner diameter of the tubing it is connected or attached to when in use. This ensures that the exhaled air pressure is maintained and there is no trapped air within the housing 102 and that the measured CO<sub>2</sub> is accurate. This also ensures that the pressure across the filter is consistently spread over the whole surface area of the filter so as not to break or pierce the filter.

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The housing 102, first end 104a, second end 104b, protrusions 106a and 106b may be made of any plastic material, and in particular a plastic material which is suitable for use in a hospital or healthcare environment. The housing 102 may be made of a recycled plastic. For example, the housing 102 may be made from upcycled syringe tips. In this regard, two syringes may be cut near their tips and joined to form the housing 102.

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The housing 102 may be separable in order to expose the interior space 114. This allows the interior space 114 to be accessed by a healthcare personnel, and thus allows additional filters or materials to be added. The housing 102 may further comprise connecting means 110. The connecting means 110 may be positioned at the separation

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interface of the housing 102. The connecting means 110 may be complementary clasps, or bayonet type connectors.

The filter device 10 may further comprises an insert 112. The insert 112 may be configured to frictionally fit with an interior wall of the housing 102. The insert 112 may be configured to frictionally fit inside the interior space 114 of the housing 102 while still allow for fluid communication between the first end 104a and second end 104b. For example, the insert 112 may comprise a through hole between its ends. The insert 112 may be further configured to engage the at least one membrane filter 108a or 108b. For example, the insert 112 may engage the membrane filter 108a or 108b at one surface, such that the other surface of the membrane filter 108a or 108b is contacted with the first end 104a and second end 104b. In this way, exhaled air from a patient must pass through the at least one membrane filter 108a or 108b. The insert 112 also prevents the exhaled air from a patient from escaping the membrane filter device 10. The insert 112 may be made from an inert material, such as Teflon.

Figure 2 shows a photograph of a capnography filter device 10 without connecting means 110. In this embodiment, the protrusions 106a and 106b are syringe nozzles. The membrane filter device 10 comprises a Teflon insert 112, the ends of which engages the first end 104a and second end 104b.

The capnography filter device 10 may be suitable dimensioned to be easily handled by healthcare personnel, while be sufficiently small to reduce cost and carbon footprint. For example, the external diameter of the housing 102 may be about 5 mm to about 15 mm and the length of the housing may be about 15 mm to about 30 mm. Preferably, the external diameter of the housing 102 may be about 9 mm and the length of the housing may be about 22 mm.

In some embodiments, the housing 102 is characterized by an outer diameter of about 8.5 mm to about 9.5 mm. In other embodiments, the outer diameter is about 8.5 mm to about 9.3 mm, about 8.5 mm to about 9.1 mm, about 8.5 mm to about 9.0 mm, about 8.8 mm to about 9.5 mm, about 8.8 mm to about 9.3 mm, about 8.8 mm to about 9.1 mm, about 8.8 mm to about 9.0 mm, about 9.0 mm to about 9.5 mm, about 9.0 mm to about 9.3 mm, or about 9.0 mm to about 9.1 mm. In some embodiments, the outer diameter is about 9.1 mm.

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In some embodiments, the housing 102 is characterized by an inner diameter of about 2.5 mm to about 4.5 mm. In other embodiments, the inner diameter is about 2.5 mm to about 4.5 mm, about 2.5 mm to about 4.3 mm, about 2.5 mm to about 4.0 mm, about 2.8 mm to about 4.5 mm, about 2.8 mm to about 4.3 mm, about 2.8 mm to about 4.0 mm, about 3.0 mm to about 4.5 mm, about 3.0 mm to about 4.3 mm, about 3.0 mm to about 4.0 mm, about 3.5 mm to about 4.5 mm, about 3.5 mm to about 4.3 mm, about 3.5 mm to about 4.0 mm, about 4.0 mm to about 4.5 mm, or about 4.0 mm to about 4.3 mm. In some embodiments, the inner diameter is about 4 mm.

In some embodiments, the housing 102 is characterized by a length of about 15 mm to 30 mm. In other embodiments, the length is about 15 mm to 22 mm, about 18 mm to 25 mm, about 18 mm to 22 mm, about 20 mm to 25 mm, or about 20 mm to 22 mm. In some embodiments, the length is about 22 mm.

The capnography filter device 10 is configured to allow an exhaled breath of a patient to flow without (or with a minimal) resistive pressure. It was found that having an interior space with a suitably sized inner capacity may reduce the pressure. It also reduces the chance of the at least one filter 108a or 108b breaking as a result of stress from the breathing pattern of the patient.

In some embodiments, the housing is characterized by an inner capacity of about 60 mm<sup>3</sup> to about 1000 mm<sup>3</sup>. In other embodiments, the inner capacity is about 60 mm<sup>3</sup> to about 900 mm<sup>3</sup>, about 60 mm<sup>3</sup> to about 800 mm<sup>3</sup>, about 60 mm<sup>3</sup> to about 700 mm<sup>3</sup>, about 60 mm<sup>3</sup> to about 600 mm<sup>3</sup>, about 60 mm<sup>3</sup> to about 500 mm<sup>3</sup>, about 60 mm<sup>3</sup> to about 450 mm<sup>3</sup>, about 60 mm<sup>3</sup> to about 400 mm<sup>3</sup>, about 60 mm<sup>3</sup> to about 350 mm<sup>3</sup>, about 60 mm<sup>3</sup> to about 300 mm<sup>3</sup>, about 60 mm<sup>3</sup> to about 250 mm<sup>3</sup>, or about 60 mm<sup>3</sup> to about 200 mm<sup>3</sup>.

In some embodiments, the housing 102 is selected from polytetrafluoroethylene, polyethylene, poly(methyl methacrylate), polyurethane, and poly(dimethylsiloxane). In some embodiments, the housing 102 is polytetrafluoroethylene.

When the capnography filter device 10 is used in capnography, the filter reduces the transfer of bacteria or viruses from the patient to the attached system and vis versa,

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thus may aid in reducing cross-contamination between patients using the same system.

To further reduce the cross-contamination, two filters 108a and 108b may be used concurrently. The two filters 108a and 108b may be spaced apart to further prevent microbes trapped on one filter from crossing to the other filter. In some embodiments, the two filters are each independently in contact with respective inner sides of the housing at the first end and second end. In this regard, the first filter is adjacent to the inner side of the first end, and the second filter is adjacent to the inner side of the second end.

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Filters work by physically trapping particles larger than the pore size and by retaining somewhat smaller particles via electrostatic attraction of the particles to the filters. Besides porosity, other factors also influence the efficiency of filtration, such as electric charge of the filter, electric charge carried by the organisms, and nature of the fluid being filtered. The at least one filter 108a or 108b may be a membrane filter, and/or HEPA filter. Membrane filter may be composed of high tensile strength polymers such as cellulose acetate, cellulose nitrate, or polysulfone. Membrane filter may have a thickness of about 150  $\mu\text{m}$  and a pore size of about 0.22  $\mu\text{m}$  to about 0.45  $\mu\text{m}$ . HEPA filter is a single sheet of borosilicate glass fiber that has been treated with a water-repellent binder. The filter may be pleated to increase the overall surface area. HEPA filters typically remove 0.3  $\mu\text{m}$  particles with an efficiency of at least 99.97% from the airstream.

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In some embodiments, the at least one filter 108a or 108b is a non-woven membrane filter layer. In some embodiments, the at least one filter is a non-woven fabric membrane filter layer. Non-woven fabrics are made in two main ways: they are either felted or bonded. The fabrics use fibres rather than yarns. The fibres are laid randomly or in a uniformed manner to form web-like layers. The fibres can be staple fibres (short) or filament fibres (continuous long) and can be bonded together by chemical, mechanical, heat or solvent treatment to form non-woven fabrics. Examples of non-woven fabrics include, but are not limited to, bamboo fabric, charcoal fabric and melt-blown polypropylene fabric. The melt blown polypropylene can further comprise charcoal, bamboo or a nano material to improve the filtration efficiency. Polytetrafluoroethylene (PTFE) membrane can also be used as it can withstand better wear and tear.

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In some embodiments, the at least one filter 108a or 108b is a N95 filter. In some  
embodiments, the at least one filter 108a or 108b is characterized by a fine particulate  
matter rating of 2.5 (PM2.5). PM2.5 refers to particulate matter which is 2.5  $\mu\text{m}$  or less  
5 in diameter. PM2.5 is generally described as fine particles. In this regard, the at least  
one filter 108a or 108b is capable of filtering particles down to 2.5  $\mu\text{m}$ .

The at least one filter 108a or 108b is porous. The porosity may regulate the flow of  
fluids (and in particular exhaled air from the patient to the capnography system).  
10 Accordingly, the pore size of the filter may be controlled in order to reduce pressure  
buildup while also be able to trap microbes. In some embodiments, the at least one  
filter 108a or 108b is capable of filtering particles with a particle size of more than 0.1  
 $\mu\text{m}$ . In other embodiments, the at least one membrane filter 108a or 108b is capable  
of filtering particles with a particle size of more than 0.2  $\mu\text{m}$ , 0.3  $\mu\text{m}$ , 0.4  $\mu\text{m}$ , 0.5  $\mu\text{m}$ ,  
15 0.6  $\mu\text{m}$ , 0.7  $\mu\text{m}$ , 0.8  $\mu\text{m}$ , 0.9  $\mu\text{m}$ , or 1  $\mu\text{m}$ .

In some embodiments, the filter 108a or 108b is characterized by a pore size of about  
0.2  $\mu\text{m}$  to about 0.5  $\mu\text{m}$ . In some embodiments, the pore size is about 0.2  $\mu\text{m}$  to about  
0.45  $\mu\text{m}$ , about 0.2  $\mu\text{m}$  to about 0.4  $\mu\text{m}$ , about 0.2  $\mu\text{m}$  to about 0.35  $\mu\text{m}$ , about 0.2  $\mu\text{m}$   
20 to about 0.3  $\mu\text{m}$ , or about 0.2  $\mu\text{m}$  to about 0.25  $\mu\text{m}$ . In some embodiments, the at least  
one filter 108a or 108b has a pore size of about 0.1  $\mu\text{m}$  to about 2.5  $\mu\text{m}$ . In other  
embodiments, the at least one filter 108a or 108b has a pore size of about 0.2  $\mu\text{m}$  to  
about 2.5  $\mu\text{m}$ , about 0.4  $\mu\text{m}$  to about 2.5  $\mu\text{m}$ , about 0.6  $\mu\text{m}$  to about 2.5  $\mu\text{m}$ , about 0.8  
 $\mu\text{m}$  to about 2.5  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 2.5  $\mu\text{m}$ , about 1.2  $\mu\text{m}$  to about 2.5  $\mu\text{m}$ , about  
25 1.4  $\mu\text{m}$  to about 2.5  $\mu\text{m}$ , about 1.6  $\mu\text{m}$  to about 2.5  $\mu\text{m}$ , about 1.8  $\mu\text{m}$  to about 2.5  $\mu\text{m}$ ,  
or about 2  $\mu\text{m}$  to about 2.5  $\mu\text{m}$ .

In addition to trapping microbes, the at least one filter 108a or 108b may also be capable  
of rendering the microbe ineffective, or killing the microbe when contacted with the  
30 surface of the membrane filter.

The at least one filter 108a or 108b may be an anti-microbial filter, an anti-viral filter,  
or a combination thereof. In some embodiments, the at least one filter comprises an  
anti-microbial composition, an anti-viral composition, or a combination thereof. An  
35 antimicrobial is an agent that kills microorganisms or stops their growth. Organic acids

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- and their salts such as lactic acid, citric acid, or acetic acid may be used as disinfectants. Heavy metal cations such as  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  have antimicrobial activities. Coordination compounds may also be used. Copper and its alloy thereof can kill microorganisms such as *E. coli* and *Staphylococcus*. The copper and alloy thereof may be used in its nanoparticle form. Many essential oils included in herbal pharmacopoeias may possess antimicrobial activity, such as the oils of bay, cinnamon, clove and thyme. Coconut oil may also be used. Quaternary Ammonium Compounds (QACs) such as benzalkonium chlorides and dodecyl dimethyl ammonium chloride can kill bacteria, viruses and mold and may be used as disinfectants. Halogen compounds such as chlorine, iodine, chloride dioxide or bromide may have different antimicrobial effects that may be influenced by pH, temperature, contact time and type of microorganism. Alcohols such as ethyl alcohol, n-propanol and isopropyl alcohol may also be used as disinfectants and antiseptics and may inhibit microorganism growth of *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus*. Phenols and phenolic compounds such as thymol, cresol, fenchlore, trischolsan, hexachlorophene may also be used as antimicrobials. Examples of other antimicrobials may include aminoglycosides, amphenicols, ansamycins, cephalosporins, cephamycins, cyclic polypeptides, glycopeptides, glycylicylines, lipopeptides, oxazolidinones, penicillins, phosphonic acid derivatives, polymyxins, quinolones, tannins, and tetracyclines.
- In some embodiments, the anti-microbial and/or anti-viral composition comprises:
- a cashew testa extract; and
  - iron-iron oxide core-shell particles, the core is an elemental iron core or an iron alloy core, and the shell is an iron oxide shell;
- wherein the cashew testa extract comprises a component selected from amino acid, protein, carbohydrate, phenolic compounds or a combination thereof; and wherein the iron-iron oxide core-shell particles are at least partially passivated by a component of the cashew testa extract.
- In some embodiments, the at least one filter 108a or 108b comprises an anti-microbial composition (iron-iron oxide composition). The iron-iron oxide composition comprises a cashew testa extract and iron-iron oxide core-shell particles. The iron core is an elemental iron core or an iron alloy core, and the shell is an iron oxide shell. The cashew testa extract comprises a component selected from protein, amino acid, sugar or carbohydrate, phenolic compounds or a combination thereof. The iron-iron oxide core-

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shell particles are at least partially passivated by a component of the cashew testa extract.

5 Certain natural products are anti-microbial. In particular, the inventors have found that an extract of cashew testa is advantageous in that it has an anti-microbial efficacy. For example, the extract was found to possess anti-bacterial properties against gram negative bacteria *Escherichia coli* and gram positive bacteria *Staphylococcus aureus*. The natural product extract can be applied in a green solvent and can have a penetrative effect when tested on surfaces and textile. Further, when combined with other  
10 components, a synergistic (or at least additive) anti-microbial effect was observed. For example, the cashew testa extract can be used as a precursor for synthesising iron particles and/or iron oxide particles. As these compositions are synthesized/made from biological or natural materials, they can be safe for human and animals. Such green biosynthesis is cost effective with less waste production, non-toxic, and environmentally  
15 friendly.

As cashew nut (*Anacardium occidentale*) is a major crop in the world and the testa (reddish brown skin) is usually discarded as waste due to its bitter and astringent taste, the testa can be collected at low cost and extracted. This extract can possess anti-  
20 bacterial properties against gram negative bacteria *Escherichia coli* and gram positive bacteria *Staphylococcus aureus*. It is advantageous to use these discarded material to synthesis an anti-microbial composition as the production cost is low and it is also environmentally green and friendly.

25 The present invention is in part predicated on the discovery that iron oxide particle formed from cashew testa extract can generate different ROS which include super oxide, singlet oxygen and hydroxyl radical. Due to the interaction of the iron oxide particles with the phenolic compounds of cashew testa extract, the ROS can be generated in the dark, i.e. in the absence of UV radiation. Further advantageously, as the ROS is  
30 continuous release by self-corrosion, an external stimulus is not required. When applied to a surface, this is beneficial for providing a constantly 'active' surface.

In particular, when the iron oxide particles are formed as iron core- iron oxide shell particles, the core-shell structure creates a special interface between iron core and iron  
35 complex shell which will change the potential of iron core and change the redox reaction

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pathway. Towards this end, it is believed that this self-corrosion process could also happen on Fe/Fe<sub>2</sub>O<sub>3</sub>, Fe/Fe<sub>3</sub>O<sub>4</sub> particles and/or a combination thereof. The electrons generated from iron corrosion can be transferred into the conduction band (CB) of iron oxide in an energetically favourable way. The electrons in the CB are able to reduce oxygen and generate ROS. In other words, the electrons are donated from iron to iron oxide (CB) and reduce oxygen molecules to generate radicals in an energetically favorable way. The whole system does not rely on an external stimulus and the ROS generation process could be manipulated and have long-term stability. ROS will then kill contacted bacteria and virus. The ROS killing mechanism of the material while being similar to photo catalyst materials, such as ZnO and TiO<sub>2</sub>, differs in that the iron/iron oxide particles is a self-catalysed material, it does not rely on photo irradiation to generate ROS. The iron particles sacrifice its iron core to generate ROS.

The ROS microbial killing mechanism is believed to be due to  $\cdot\text{O}_2^-$ ,  $\text{H}_2\text{O}_2$ ,  $\cdot\text{OH}$ ,  $^1\text{O}_2$  and/or  $\alpha\text{-O}$ .

In some embodiments, the ROS can be generated in the dark. In other embodiments, the ROS can be generated in the absence of UV radiation. In other embodiments, the ROS generated is selected from  $\cdot\text{O}_2^-$ ,  $\text{H}_2\text{O}_2$ ,  $\cdot\text{OH}$ ,  $^1\text{O}_2$ ,  $\alpha\text{-O}$ , or a combination thereof.

It is postulated that the membrane filter will trap the microbes by mimicking the sites on human cells to which they normally attach, and then destroy them by disrupting their surfaces (viruses) and cell walls (bacteria). It was found that the membrane filter can kill germs which can cause Influenza A, Bird Flu, SARS, measles, pneumonia, common colds, tuberculosis, herpes, MRSA and gastroenteritis.

The anti-bacterial activity is believed to be via a mechanism in which proteins of microbial membranes can be bound to the composite material, which damages the bacterial cell's structure and function. Further complexation with essential metal ions also inhibits fibrin formation.

Further, anti-viral activity can also be effected. The membrane filter can attack different stages of the viral replication process. This includes the extracellular virions themselves, during the attachment of the virus to the cell, during the penetration of the virus into the cell, during the viral replication process in the host cell, as well during the assembly

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of new viral particles, transport proteins, polysaccharides, and viral enzymes. In almost all of the abovementioned stages, the composite will bind permanently to the proteins of the capsid or supercapsid. The proteins may be either the specific viral enzymes required for viral replication or to the newly synthesized viral proteins that are involved  
5 in the production of the new viral particle.

The presently disclosed device is thus effective in its anti-microbial function and may have a long shelf-life. Inorganic antimicrobial materials, especially semiconductor antimicrobial materials are less prone to chemical contamination and possess long-term  
10 stability. Some metal or metal oxides, such as silver, zinc oxide and titanium oxide particles have been used as antimicrobial ingredients in various products or in antimicrobial surface coatings. However, these materials also have limitations such as heavy metal contamination/toxicity (for Ag based materials). For ZnO and TiO<sub>2</sub> materials, they suffer from low anti-microbial efficacies and limited applications, due to  
15 the dependence on photo irradiation. In addition, uncertainty nano-toxicity is another concern for nano-size materials.

Advantageously, the iron based antimicrobial composition is non-toxic, yet can be highly active against microbes, very stable and has long-term activity. For example, the iron-  
20 iron oxide composition can be synthesized by modifying iron powder (microns size) with carbohydrates, amino acids, food additives or nutrition, under non organic solvent conditions. This can be done, for example, using a fluidised bed reactor. Iron powder is redox active, it will slowly react with oxygen and moisture to form iron oxides and releasing hydrogen. Iron powder itself will not generate reactive oxygen species (ROS)  
25 and does not kill bacteria. There could be some iron cation releasing out from iron powder, but it is in very low concentration and not harmful to cells. By modifying iron powder (microns size) with carbohydrates, amino acids, food additives or nutrition, the iron particles can have a nano-structured protection shell covered the iron core. This shell can be formed by the reaction of iron with carbohydrates, amino acids, food  
30 additives under non organic solvent conditions. The core-shell structure creates a special interface between iron core and iron complex shell which changes the potential of iron core and the redox reaction pathway. Accordingly, the iron particles can react with oxygen and moisture to generate ROS. Iron oxides and hydroxides are kept in the core part. In this way, the iron particles can generate different ROS which include super  
35 oxide, singlet oxygen and hydroxyl radical. ROS can then kill contacted bacteria and

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virus. The ROS killing mechanism of the material is similar to photo catalyst materials, such as ZnO and TiO<sub>2</sub>. The ROS generation mechanism of this material is different from those photo catalyst materials. It is self-catalysed materials, it does not rely on photo irradiation to generate ROS. The iron particles sacrifices iron core to generate ROS.

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The cashew testa extract may comprise other components, such as protein, amino acid, starch, sugar, carbohydrate, phenolic compounds or a combination thereof, the phenolic compounds selected from tannins, catechin, epicatechin, epigallocatechin, p-coumaric, gallic acid, or a combination thereof.

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In some embodiments, the amino acid relative to the iron-iron oxide composition is at about 2% w/w to about 6% w/w. In other embodiments, the amino acid relative to the iron-iron oxide composition is at about 2% w/w to about 5% w/w, or about 3% w/w to about 5% w/w.

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The term "amino acid" is defined as having at least one primary, secondary, tertiary or quaternary amino group, and at least one acid group, wherein the acid group may be a carboxylic, sulfonic, or phosphonic acid, or mixtures thereof. The amino groups may be "alpha", "beta", "gamma" ... to "omega" with respect to the acid group(s). The backbone of the "amino acid" may be substituted with one or more groups selected from halogen, hydroxy, guanido, heterocyclic groups. Thus term "amino acids" also includes within its scope glycine, alanine, valine, leucine, isoleucine, methionine, proline, phenylalanine, tryptophane, serine, threonine, cysteine, tyrosine, asparagine, aspartic acid, glutamine, glutamic acid, lysine, arginine, histidine, taurine, betaine, N-methylalanine, etc. (L) and (D) forms of amino acids are included in the scope of the present disclosure.

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In some embodiments, the protein relative to the iron-iron oxide composition is at about 2% w/w to about 6% w/w. In other embodiments, the protein relative to the iron-iron oxide composition is at about 2% w/w to about 5% w/w, or about 3% w/w to about 5% w/w.

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"Polypeptide", "peptide" and "protein" are used interchangeably herein to refer to any polymer of amino acid residues (dipeptide or greater) linked through peptide bonds or modified peptide bonds and to variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues is

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a synthetic non-naturally occurring amino acid, such as a chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally-occurring amino acid polymers. Polypeptides of the present invention include, but are not limited to, naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. The polypeptides of the invention may comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a polypeptide by the cell in which the polypeptide is produced, and will vary with the type of cell. For polypeptides that are made recombinantly, the nature and extent of the modifications in large part will be determined by the post-translational modification capacity of the particular host cell and the modification signals that are present in the amino acid sequence of the polypeptide in question. For instance, glycosylation patterns vary between different types of host cell. Polypeptides are defined herein, in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Proteins may be present as monomeric or as multimeric proteins e.g. as dimers (homo or heterodimers) or trimers.

In some embodiments, the carbohydrate relative to the iron-iron oxide composition is at about 2% w/w to about 6% w/w. In other embodiments, the carbohydrate relative to the iron-iron oxide composition is at about 2% w/w to about 5% w/w, or about 3% w/w to about 5% w/w. In some embodiments, the carbohydrate relative to the iron-iron oxide composition is at about 2% w/w to about 20% w/w.

As used herein, a carbohydrate is a biomolecule consisting of carbon (C), hydrogen (H) and oxygen (O) atoms, usually with a hydrogen-oxygen atom ratio of 2:1 (as in water) and thus with the empirical formula  $C_m(H_2O)_n$  (where m may be different from n). However, not all carbohydrates conform to this precise stoichiometric definition (e.g., uronic acids, deoxy-sugars such as fructose). The term is a synonym of saccharide, a group that includes sugars, starch, and cellulose. The saccharides are divided into four chemical groups: monosaccharides, disaccharides, oligosaccharides, and polysaccharides. Monosaccharides and disaccharides, the smallest (lower molecular weight) carbohydrates, are commonly also referred to as sugars. Examples of

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carbohydrates or sugars are monosaccharides such as glucose, galactose, fructose, xylose; disaccharides such as sucrose, lactose, maltose, trehalose, polyols such as sorbitol, mannitol; oligosaccharides such as malto-oligosaccharides (maltodextrins), raffinose, stachyose, fructo-oligosaccharides; polysaccharides such as starch (amylose, amylopectin, modified starches) and non-starch polysaccharides (glycogen, cellulose, hemicellulose, pectins, hydrocolloids).

The carbohydrate can be selected from monosaccharide, disaccharide, oligosaccharide, and polysaccharide. Examples of carbohydrate are, but not limited to, glucose, galactose, fructose, xylose, sucrose, lactose, maltose, trehalose, sorbitol, mannitol, maltodextrin, raffinose, stachyose, fructo-oligosaccharide, amylose, amylopectin, modified starch, glycogen, dextran, chitosan, glycosaminoglycans, alginate, ulvan, gum Arabic, gellan gum, cellulose, hemicellulose, ethylcellulose, methylcellulose, pectin, hydrocolloid and a combination thereof.

In other embodiments, the iron-iron oxide composition further comprises an amino acid. In other embodiments, the iron-iron oxide composition further comprises an amino acid, carbohydrate or a mixture thereof. In some embodiments, the amino acid, carbohydrate or a mixture thereof relative to the iron-iron oxide composition is at about 2% w/w to about 6% w/w. In other embodiments, the amino acid, carbohydrate or a mixture thereof relative to the iron-iron oxide composition is at about 2% w/w to about 5% w/w, or about 3% w/w to about 5% w/w. For example, a mixture of methylcellulose and zein can be used.

Cashew testa extract contains many polyphenols, including tannic acid. Tannic acid is a water soluble and reddish brown coloured molecule. It is believed that tannic acid, and in general phenolic compounds, can have an anti-microbial effect by binding to the bacteria, disrupt the bacteria's cell membrane integrity and also disrupt various functions inside the bacteria cell.

One hypothesis is that changes in various intracellular functions induced by hydrogen binding of the phenolic compounds to enzymes or by the modification of the cell wall rigidity with integrity losses due to different interactions with the cell membrane. This may induce irreversible damages of the cytoplasmic membrane and coagulation of the cell content that can even lead to the inhibition of intracellular enzymes. For example,

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condensed phenylpropanoids—tannins may induce damages at the cell membrane and even inactivate the metabolism by binding to enzymes while phenolic acids have been shown to disrupt membrane integrity, as they cause consequent leakage of essential intracellular constituents. Flavonoids may link to soluble proteins located outside the cells and with bacteria cell walls thus promoting the formation of complexes. Flavonoids also may act through inhibiting both energy metabolism and DNA synthesis thus affecting protein and RNA syntheses. In the case of Gram-positive bacteria, intracellular pH modification as well as interference with the energy (ATP) generating system were reported.

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In some embodiments, the composition comprises polyphenols or phenolic compounds such as tannins, catechin, epicatechin, epigallocatechin, and p-coumaric, gallic acid, or a combination thereof. These polyphenols possess high antioxidant and demonstrate high free radical scavenging activity. The compounds of the phenolic compounds can be in any desired percentages or ratios. The phenolic compounds as used herein refer to chemical compounds that contain at least one aromatic ring with hydroxyl groups (-OH) attached.

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In some embodiments, the phenolic compounds relative to the iron-iron oxide composition is at about 2% w/w to about 6% w/w. In other embodiments, the phenolic compounds relative to the iron-iron oxide composition is at about 2% w/w to about 5% w/w, or about 3% w/w to about 5% w/w.

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In some embodiments, a weight ratio of phenolic compounds to protein is about 1:100 to about 100:1.

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In some embodiments, a weight ratio of phenolic compounds to sugar is about 1:100 to about 100:1.

In some embodiments, the iron-iron oxide core-shell particles are at least partially passivated by phenolic compounds. In some embodiments, the iron-iron oxide core-shell particles are at least partially passivated by protein, amino acid or a combination thereof. In some embodiments, the iron-iron oxide core-shell particles are at least partially passivated by sugar, carbohydrate or a combination thereof.

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Advantageously, it was found that the physical combination of cashew testa extract with iron particles and/or iron oxide particles act synergistically (or at least additively) on each other such that the anti-microbial effect is increased. Without wanting to be bound by theory, it is believed that when the compounds in cashew testa extract are allowed to passivate the iron/iron oxide particles, aggregation and/or agglomeration of iron/iron oxide particles can be decreased. Further, as the stability of the particles are improved, it was found that when applied to a porous substrate or textile, the composition can impregnate the surface of the porous substrate. This is in contrast to cashew testa extract alone or iron/iron oxide particles alone, where they are found to reside at the surface. Accordingly, the retention time of the composition on the surface is improved, and the anti-microbial efficacy is correspondingly increased.

At least the phenolic compounds when adsorbed on the surface of the iron/iron oxide particles were found to be slightly protected from degraded, hence ensuring that the anti-microbial effect is longer lasting.

In some embodiments, the iron-iron oxide composition comprises iron, iron (II) oxide and iron (III) oxide. In some embodiments, the iron relative to the iron-iron oxide composition is at more than 90% w/w. In other embodiments, the iron relative to the iron-iron oxide composition is at more than 91% w/w, more than 92% w/w, more than 93% w/w, more than 94% w/w, more than 95% w/w, more than 96% w/w, or more than 97% w/w. In other embodiments, the iron (II) oxide and iron (III) oxide relative to the iron-iron oxide composition is at less than 10% w/w. In other embodiments, the iron (II) oxide and iron (III) oxide relative to the iron-iron oxide composition is at less than 9% w/w, at less than 8% w/w, at less than 7% w/w, at less than 6% w/w, at less than 5% w/w, at less than 4% w/w, or at less than 3% w/w.

In some embodiments, the iron is elemental iron. As disclosed herein, at least a surface of the iron particles is oxidized to iron oxide. In other embodiments, the iron (II) oxide is FeO. In other embodiments, the iron (III) oxide is Fe<sub>2</sub>O<sub>3</sub>. In other embodiments, the iron (II) oxide and iron (III) oxide is Fe<sub>3</sub>O<sub>4</sub>. In this regard, the iron-iron oxide composition can be a mixture of Fe, FeO, Fe<sub>2</sub>O<sub>3</sub> and Fe<sub>3</sub>O<sub>4</sub>.

In some embodiments, the iron-iron oxide core-shell particles are at least partially passivated by a component of the cashew testa extract. In contrast to directly adding

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cashew testa extract as a coating layer on the filter, it was found that by mixing in cashew testa extract during the synthesis of iron particles, the cashew testa extract can at least partially passivate the surface of iron particles. This further improves the stability of the iron particles as well as controls the release of ROS from the iron particles. Further advantageously, by reacting cashew testa extract with iron particles and/or iron oxide precursor, in addition to the partially passivate by the cashew testa extract, the cashew testa extract also forms part of a shell covering the iron particle such that the release of ROS can be extended for a longer duration.

10 Towards this end, the inventors have found that the anti-microbial efficacy can be further improved synergistically (or at least additively) through the combination of cashew testa extract and iron particles and/or iron oxide particles. Advantageously, the inventors have found that an iron based antimicrobial material is non-toxic, yet can be highly active against microbes, very stable and has long-term activity.

15 In some embodiments, the iron-iron oxide composition comprises iron,  $\text{Fe}_3\text{O}_4$  and an amino acid. In some embodiments, the iron relative to the iron-iron oxide composition is at more than 95% w/w, the  $\text{Fe}_3\text{O}_4$  relative to the iron-iron oxide composition is at less than 2% w/w, and the amino acid relative to the iron-iron oxide composition is at about 3% w/w to about 5% w/w.

20 In some embodiments, the iron-iron oxide composition comprises iron,  $\text{Fe}_3\text{O}_4$  and a carbohydrate. In some embodiments, the iron relative to the iron-iron oxide composition is at more than 95% w/w, the  $\text{Fe}_3\text{O}_4$  relative to the iron-iron oxide composition is at less than 2% w/w, and the carbohydrate relative to the iron-iron oxide composition is at about 3% w/w to about 5% w/w.

25 In some embodiments, the iron-iron oxide composition comprises iron,  $\text{Fe}_3\text{O}_4$ , and amino acid, carbohydrate or a mixture thereof. In some embodiments, the iron relative to the iron-iron oxide composition is at more than 95% w/w, the  $\text{Fe}_3\text{O}_4$  relative to the iron-iron oxide composition is at less than 2% w/w, and the amino acid, carbohydrate or a mixture thereof relative to the iron-iron oxide composition is at about 3% w/w to about 5% w/w.

35 As used herein, 'particles' refer to micron-sized particles and/or nano-sized particles.

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Microparticles are particles between 1 and 1000  $\mu\text{m}$  in size. Nanoparticles are particles between 1 nm to 1000 nm in size. The particles can be of any shape or morphology, such as spherical, rod-like, or asymmetrical.

5 In some embodiments, the iron-iron oxide composition is provided as a powder with a particle size of about 1  $\mu\text{m}$  to about 100  $\mu\text{m}$ . In some embodiments, the iron-iron oxide composition has a particle size of about 1  $\mu\text{m}$  to about 800  $\mu\text{m}$ . In this regard, the iron-iron oxide can be micron sized particles. In other embodiments, the particle size is about 1  $\mu\text{m}$  to about 700  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 600  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 500  $\mu\text{m}$ ,  
10 about 1  $\mu\text{m}$  to about 400  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 300  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 200  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 100  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 90  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 80  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 70  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 60  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 50  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 40  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 30  $\mu\text{m}$ , or about 10  $\mu\text{m}$  to about 40  $\mu\text{m}$ .

15 The iron-iron oxide composition is present relative to the at least one filter 108a or 108b at about 0.1% w/w to about 5% w/w. In other embodiments, the concentration is at about 0.1% w/w to about 4% w/w, at about 0.1% w/w to about 3% w/w, at about 0.1% w/w to about 2% w/w, at about 0.1% w/w to about 1% w/w, or at about 0.1% w/w to  
20 about 0.5% w/w. In other embodiments, the concentration is less than 5% w/w, less than 4% w/w, less than 3% w/w, less than 2% w/w, less than 1% w/w, less than 0.5% w/w, or less than 0.2% w/w.

In some embodiments, the iron-iron oxide composition is impregnated/present relative  
25 to the filter at about 0.1% w/w to about 5% w/w. In this regard, the dry mass of the iron-iron oxide composition in relation to the final membrane filter (combination of filter and iron-iron oxide composition) dry mass is about 0.1% w/w to about 5% w/w. In other embodiments, the impregnation is at about 0.1% w/w to about 4% w/w, at about 0.1% w/w to about 3% w/w, at about 0.1% w/w to about 2% w/w, at about 0.1% w/w  
30 to about 1% w/w, or at about 0.1% w/w to about 0.5% w/w. In other embodiments, the impregnation is less than 5% w/w, less than 4% w/w, less than 3% w/w, less than 2% w/w, less than 1% w/w, less than 0.5% w/w, or less than 0.2% w/w.

As used herein, 'impregnate' means to soak or saturate (something) with a substance.  
35 In this regard, the membrane filter is soaked with the iron-iron oxide composition.

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Advantageously, the impregnation of the iron-iron oxide composition in the porous fabric allows for a homogenous dispersion of the iron particles in the membrane filter. In this regard, the iron-iron oxide composition is retained in the membrane filter.

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In use, moisture or water vapour from a patient's exhaled breath may pass through the membrane filter. This may further 'activate' the iron particles. The iron, in the presence of oxygen and water, may be converted to iron oxide and in the process releases ROS. In this regard, a reservoir of ROS is constantly available.

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Advantageously, when the iron particles are encapsulated with a shell, the iron core can be protected by an encapsulation shell material. This may further delays the production of ROS until its use. This prevents or reduces over oxidation of iron particles, and thus allows for a longer shelf life and/or create a further persistence ROS over a longer period of time. Further, the amino acid and/or carbohydrate encapsulation material can change the potential of iron core and change the redox reaction pathway. For example, the iron oxide generated can form an interface layer between the shell and the Fe core. This allows the generation and release of ROS to be controlled. In this way, the shell on the iron particles controls the rate of iron oxidation, such that a constant release of ROS is available, which is sufficient for the anti-bacterial and/or anti-viral effect. This improves its suitability for multiple uses, allowing for additional washing. A further advantage is that as a natural compound such as a biopolymer is used to encapsulate the iron particle, the biodegradable nature of the biopolymer causes the shell to break down over time. For example, the shell can be broken down over time after multiple washing. This provides an additional anti-microbial and/or anti-viral persistence effect, as the previously less accessible inner iron core can now be more easily accessed.

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In some embodiments, the shell has a thickness of about 50 nm to about 400 nm. In other embodiments, the thickness is of about 50 nm to 350 nm, about 50 nm to about 300 nm, about 100 nm to about 300 nm, about 150 nm to about 300 nm, or about 200 nm to about 300 nm.

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The iron-oxide shell may also interact chemically with the cashew testa extract. The anti-microbial efficacy of the composition may thus be further improved. In some embodiments, the shell further comprises at least a component of the cashew testa

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extract. In this regard, the shell comprises iron oxide and at least a component of the cashew testa extract. The iron oxide may form an interface between the iron core and the cashew testa extract component. Alternatively, the shell may comprise a homogenous composition of iron oxide and cashew testa extract component. The cashew testa extract component may be an amino acid, carbohydrate, phenolic compound protein, sugar or a combination thereof. In some embodiments, the iron oxide is  $\text{Fe}_3\text{O}_4$ . In some embodiments, the amino acid is selected from glycine, alanine, valine, leucine, isoleucine, methionine, proline, phenylalanine, tryptophane, serine, threonine, cysteine, tyrosine, asparagine, aspartic acid, glutamine, glutamic acid, lysine, arginine, histidine, taurine, betaine, N-methylalanine or a combination thereof.

Advantageously, by allowing the cashew testa extract to react with the iron particles, an activated shell of iron oxide and cashew testa extract can be formed on a core of elemental iron or iron alloy. The thickness of the iron oxide shell may be controlled by the reaction conditions as well as the amount of cashew testa extract to iron powder ratio. The formation of a cashew-iron oxide shell provides for a greater anti-microbial efficacy as the retention time is longer and the surface area of contact is increased. Additionally, it was found that having a core of elemental iron or iron alloy is advantageous as it regenerates the outer iron oxide shell as the shell gets 'used up'.

In other embodiments, the iron-iron oxide composition may comprise a plurality of micron sized particles, the particles having an iron core encapsulated with an organic shell. The organic shell may be formed from the cashew testa extract. The shell may comprise an amino acid, carbohydrate or a mixture thereof. In some embodiments, the shell comprises an amino acid. In some embodiments, the shell comprises a carbohydrate.

Amino acids can be used as encapsulation materials. In such examples, the amino acids can be polymerised into a peptide. Peptides can also be used as encapsulation materials. Such are also included within the definition of 'amino acid'. For example, zein can be used.

Similarly to carbohydrates, when monosaccharides and/or disaccharides are used, these saccharides can be polymerised during the encapsulation process. Longer chain biopolymer can result, which creates a shell around the particle. Accordingly,

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monosaccharides, disaccharides, oligosaccharides and polysaccharides are included within the definition of 'carbohydrate'. For example, cyclodextrins can be used.

5 The shell may further comprise iron. In this regard, in some embodiments, the shell comprises iron and an amino acid, carbohydrate or a mixture thereof. Advantageously, as the iron is closer to the surface of the particles, the presence of iron in the shell can 'kick starts' the oxidation of iron to iron oxide. In this sense, a burst release of ROS is provided at first instance, which can provide protection to a user when the face mask is first used and before sufficient water is provided as moisture to the iron core.

10 In some embodiments, the iron-iron oxide composition is a plurality of iron core-shell micron sized particles, wherein the core comprises Fe and the shell comprises Fe and an amino acid. In other embodiments, the iron-iron oxide composition is a plurality of iron core-shell micron sized particles, wherein the core comprises Fe, the shell  
15 comprises Fe and an amino acid, and an interface between the core and the shell comprises iron oxide. In some embodiments, the iron oxide is Fe<sub>3</sub>O<sub>4</sub>. In some embodiments, the amino acid is selected from glycine, alanine, valine, leucine, isoleucine, methionine, proline, phenylalanine, tryptophan, serine, threonine, cysteine, tyrosine, asparagine, aspartic acid, glutamine, glutamic acid, lysine, arginine, histidine,  
20 taurine, betaine, N-methylalanine or a combination thereof.

In some embodiments, the iron-iron oxide composition further comprises an excipient. The excipient can be a stabilising agent, dispersant, colorant, drying agent or a combination thereof.

25 "Excipients" are inactive substances that serve as the vehicle or medium for an active substance, and include any and all solvents, dispersion media, inert diluents, or other liquid vehicles, dispersion or suspension aids, granulating agents, surface active agents, disintegrating agents, isotonic agents, thickening or emulsifying agents, preservatives,  
30 binding agents, lubricants, buffering agents, oils, and the like. Various excipients used in formulating compositions and known techniques for the preparation thereof is disclosed in G. A. R. Remington: The Science and Practice of Pharmacy, 21st ed. (2006), Lippincott Williams & Wilkins. Except insofar as any conventional excipient is incompatible with a substance or its derivatives, such as by producing any undesirable  
35 biological effect or otherwise interacting in a deleterious manner with any other

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component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention.

5       Excipients such as colouring agents, coating agents, preservatives and perfuming agents can be present in the composition, according to the judgment of the formulator. Examples of excipients are colloidal silica, hydroxypropyl methylcellulose, vitamin A, vitamin E, vitamin C, retinyl palmitate, selenium, sodium metabisulphite, propyl gallate, cysteine, methionine, citric acid, sodium citrate, methyl paraben, propyl paraben, benzalkoniumchloride, and lanolin.

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      In some embodiments, the excipient is selected from a surfactant, a stabiliser and/or polymers. Such excipients can act to improve the dispersibility of the iron particles in the application medium. Surfactants are compounds that lower the surface tension (or interfacial tension) between two liquids, between a gas and a liquid, or between a liquid  
15       and a solid. Examples of surfactants are sodium lauryl sulfate (SDS), cetyl trimethylammonium bromide (CTAB), Triton X-100, X-114, CHAPS, DOC, NP-40, octyl thioglucoside, octyl glucoside and dodecyl maltoside. A stabilizer or stabiliser is a chemical that is used to prevent degradation due to, for example, heat and/or light. For example, antioxidants, such as oxygen scavengers, persistent radical scavengers and  
20       antiozonants can be added to further control the rate of generation of ROS (as disclosed herein). Sequestrants can also be added to form chelates complexes and inactivate trace metal ions on surfaces that would otherwise act as catalyst or inactivate the composition. UV stabilisers (UV absorbers and quenchers) can also be added to protect the composition from degradation. Polymers which includes polyelectrolytes such as  
25       polyacrylic acid (PAA), poly(sodium styrene sulfonate) (PSS), polyethylenimine (PEI), poly(lactide-co-glycolide) (PLGA), and fluoropolymers can be added to improve application onto surfaces as well as increase the retention time of the ROS.

      Advantageously, this allows for the ease of transport and storage of the composition.  
30       The shelf-life of the composition can also be further improved.

      A drying agent may be used to remove moisture from the exhaled air of the patient. This may provide further protection to the capnography instrument, and further improve the readout. Examples of desiccants are calcium chloride, calcium sulfate, activated  
35       carbon, zeolites and silica gel.

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The membrane filter may further comprise a water repellent coating. The coating may be formed from perfluorobutanesulfonic acid, perfluorooctanoic acid, perfluorohexanoic acid, Scotchgard, perfluorooctanesulfonic acid, paraffin (and other hydrocarbon based solutions), silica nanoparticles and silanes (e.g. alkyl trialkoxy silanes).

Advantageously, the water repellent coating prevents or reduces the oxidation of the iron particles during washing. As mentioned herein, iron is oxidised to iron oxide in the presence of water and oxygen. It was advantageously found that the water repellent coating can further provide protection to the iron-iron oxide composition, by acting synergistically with the shell on the iron particle. In this regard, better control over the release of ROS can be achieved.

In some embodiments, the water repellent coating is on one side of the membrane filter. When coated on one side of the membrane filter, the coating can be on the side that is impregnated with iron-iron oxide composition. In some embodiments, the water repellent coating is on at least one side of the membrane filter. For example, the coating may be formed on an inner side of the membrane filter, facing the interior space 114 of the housing 102. In other embodiments, the water repellent coating is on both sides of the porous fabric.

In some embodiments, the water repellent coating has a thickness of about 1  $\mu\text{m}$  to about 1000  $\mu\text{m}$ . In other embodiments, the thickness is of about 1  $\mu\text{m}$  to about 900  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 800  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 700  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 600  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 500  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 400  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 300  $\mu\text{m}$ , about 1  $\mu\text{m}$  to about 200  $\mu\text{m}$ , or about 1  $\mu\text{m}$  to about 100  $\mu\text{m}$ . In other embodiments, the thickness is of about 10  $\mu\text{m}$  to about 1000  $\mu\text{m}$ , about 10  $\mu\text{m}$  to about 900  $\mu\text{m}$ , about 10  $\mu\text{m}$  to about 800  $\mu\text{m}$ , about 10  $\mu\text{m}$  to about 700  $\mu\text{m}$ , about 10  $\mu\text{m}$  to about 600  $\mu\text{m}$ , about 10  $\mu\text{m}$  to about 500  $\mu\text{m}$ , about 10  $\mu\text{m}$  to about 400  $\mu\text{m}$ , about 10  $\mu\text{m}$  to about 300  $\mu\text{m}$ , about 10  $\mu\text{m}$  to about 200  $\mu\text{m}$ , or about 10  $\mu\text{m}$  to about 100  $\mu\text{m}$ .

In some embodiments, the membrane filter further comprises a second coating, the second coating formed from an extract obtained from cashew testa. This can be in addition to the cashew testa extract which is present in the iron-iron oxide composition.

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Advantageously, the cashew testa extract coating provides an additional protective layer further to the iron-iron oxide composition. Cashew testa extract contains many polyphenols, including tannic acid. Tannic acid is a water soluble and reddish brown coloured molecule. The antimicrobial property of tannic acid can also be effective against the growth of many fungi, yeasts, gram positive bacteria such as *Staphylococcus aureus* and viruses (noroviruses, the influenza A virus, and papilloma viruses) by the anti-bacterial and anti-viral mechanisms as disclosed herein.

In some embodiments, the cashew testa extract coating has a thickness of about 10  $\mu\text{m}$  to about 1000  $\mu\text{m}$ . In other embodiments, the thickness is of about 10  $\mu\text{m}$  to about 900  $\mu\text{m}$ , about 10  $\mu\text{m}$  to about 800  $\mu\text{m}$ , about 10  $\mu\text{m}$  to about 700  $\mu\text{m}$ , about 10  $\mu\text{m}$  to about 600  $\mu\text{m}$ , about 10  $\mu\text{m}$  to about 500  $\mu\text{m}$ , about 10  $\mu\text{m}$  to about 400  $\mu\text{m}$ , about 10  $\mu\text{m}$  to about 300  $\mu\text{m}$ , about 10  $\mu\text{m}$  to about 200  $\mu\text{m}$ , or about 10  $\mu\text{m}$  to about 100  $\mu\text{m}$ . In some embodiments, the cashew testa extract coating is coated on at least one side of the membrane filter. In other embodiments, the cashew testa extract coating is coated on both sides of the membrane filter.

The iron-iron oxide composition may further comprise particles with a nano-structured protection shell covering the iron core. This shell may be formed by the reaction of iron and cashew testa extract under non organic solvent conditions. The core-shell structure creates a special interface between iron core and iron complex shell which will change the potential of iron core and change the redox reaction pathway. Towards this end, it is believed that this self-corrosion process could also happen on Fe/Fe<sub>2</sub>O<sub>3</sub>, Fe/Fe<sub>3</sub>O<sub>4</sub> particles and/or a combination thereof. The electrons generated from iron corrosion can be transferred into the conduction band (CB) of iron oxide in an energetically favourable way. The electrons in the CB are able to reduce oxygen and generate ROS. The iron particles could generate different ROS which include super oxide, singlet oxygen and hydroxyl radical. In other words, the electrons are donated from iron to iron oxide (CB) and reduce oxygen molecules to generate radicals in an energetically favorable way. The whole system does not rely on an external stimulus and the ROS generation process could be manipulated and have long-term stability. ROS will then kill contacted bacteria and virus. The ROS killing mechanism of the material while being similar to photo catalyst materials, such as ZnO and TiO<sub>2</sub>, differs in that the iron/iron oxide particles is a self-catalysed material, it does not rely on photo irradiation to generate ROS. The iron particles sacrifices its iron core to generate ROS. New materials designed based on this

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concept could play pivotal roles as non-toxic and safe antimicrobial technology to replace organic disinfectants, antiseptics and antibiotics in a broad range of applications, especially in the control of infectious disease and antimicrobial resistance (AMR) transmission.

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Without wanting to be bound by theory, the inventors believe that iron oxide nanoparticles may be synthesised through plant-mediated green chemistry approach via using plant extract as a reducing agent and a metal precursor under suitable conditions. This process consists of three steps: (1) the activation phase in which the metal ions are reduced by the phenolic compounds in the plant extract followed by the nucleation of reduced metal atoms; (2) the growth phase, where small NPs adhere to form large sized NPs (Ostwald ripening); and (3) the termination phase, during which NPs attain their shape. The phenolic compounds can also act as stabilizing agents, capping the surface of the nanoparticles.

15

Advantageously, by allowing the cashew testa extract to react with the iron oxide precursor, an activated iron oxide and cashew testa extract nanoparticle can be formed. In this method, the whole of the nanoparticle is activated. The size of the iron oxide nanoparticles can be controlled by the reaction conditions as well as the amount of cashew testa extract to iron oxide precursor ratio. Activity is found to be greater for these nanoparticles due to the increased surface area and surface energy, which favours an equilibrium towards dissolution.

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In other embodiments, the iron oxide nanoparticles comprises an iron-cashew testa extract complex. In other embodiments, the iron oxide nanoparticles comprises an iron-phenolic compound complex. To this end, the nanoparticle is made up of network or matrix of iron atoms and phenolic compounds (or at least carbon atoms).

25

In some embodiments, the iron oxide nanoparticles are at least partially passivating by the phenolic compounds. Towards this end, the unreacted cashew testa extract can physically adsorb on the surface of the iron oxide nanoparticles through like-like interactions. This provides for greater stability of the composition, and hence shelf-life. In some embodiments, a volume ratio or weight ratio of the cashew testa extract to iron oxide particle precursor is about 100:1 to about 1:100. In other embodiments, the ratio is about 90:1, 80:1, 70:1, 60:1, 50:1, 40:1, 30:1, 20:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1,

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4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80, or 1:90.

5 In some embodiments, the iron oxide nanoparticles has an average size of about 1 nm to about 1000 nm. In other embodiments, the average size is about 10 nm to about 50 nm.

10 In some embodiments, the shell is a homogenous shell. As used herein, 'homogenous' refers to the shell having a same proportions of its components throughout. In this regard, the shell is an iron oxide layer in which cashew testa extract is homogeneously dispersed within the layer (or at least at an interface). The shell can also be of a homogenous thickness throughout. For example, the thickness measured at various portions of a single iron oxide particle can vary at (or have a standard deviation of) less than 30%, less than 20% or less than 10%.

15 In some embodiments, the shell is an inhomogeneous shell. For example, the shell may be formed by first forming the iron oxide nanoparticles (in the presence of cashew testa extract) and subsequently adhering the nanoparticles on the larger iron powder to give a particle with patchy shell of iron oxide on an iron core is formed. Because of the patchiness of the shell, the activity of the iron oxide is increased, while the problem of aggregation of nanoparticles is minimised. Further, as the nanoparticles are anchored to a heavier and larger particle, the retention on a surface is improved; i.e. it is less easily washed away.

25 As used herein, 'patchy particles' or 'patchy nanoparticles' are micron- or nanoscale colloidal particles that are anisotropically patterned. This can be either by modification of the particle surface chemistry ("enthalpic patches"), through particle shape ("entropic patches"), or both.

30 In some embodiments, the at least one filter 108a or 108b is characterized by a filtration efficiency for bacterial and/or viruses of at least about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 98%. In other embodiments, the at least one membrane filter 108a or 108b is capable of filtering particles with a size of more than 0.3  $\mu\text{m}$  and with an efficiency of at least about 80%.

35 In other embodiments, the at least one membrane filter 108a or 108b is capable of

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filtering particles with a size of about 0.1  $\mu\text{m}$  and with an efficiency of at least about 80%. Accordingly, when two membrane filters 108a and 108b are used, the efficiency may be improved.

- 5 In some embodiments, the at least one filter 108a or 108b has a bacterial filtration efficiency of at least 90% after 30 days. In other embodiments, the bacterial filtration efficiency is at least 98%, at least 96%, at least 94%, at least 92%, at least 90%, at least 85% at least 80% at least 75% at least 70%, at least 65%, or at least 60%.
- 10 In some embodiments, the at least one membrane filter 108a or 108b has an anti-bacterial efficacy of at least 90% after 24 h. In other embodiments, the anti-bacterial efficacy is at least 98%, at least 96%, at least 94%, at least 92%, at least 90%, at least 85% at least 80% at least 75% at least 70%, at least 65%, or at least 60%.
- 15 In some embodiments, the device has an anti-bacterial activity of at least 5 log reduction. In other embodiments, the device has an anti-bacterial activity of at least 4 log reduction, at least 3 log reduction, at least 2 log reduction or at least 1 log reduction. The device has an anti-bacterial activity against *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 15442, *Enterococcus faecium* ATCC 6057, *Salmonella typhimurium* ATCC 13311.
- 20

- In some embodiments, the at least one membrane filter 108a or 108b has an anti-viral efficacy with at least 0.5-log unit inhibition after 24 h. The efficacy can be compared against a control. In other embodiments the anti-viral inhibition is at least 1-log unit, at
- 25 least 1.5-log unit, at least 2-log unit, at least 2.5-log unit, at least 3-log unit, at least 3.5-log unit, at least 4-log unit, or at least 4.5-log unit.

- In some embodiments, the at least one membrane filter is characterized by a diameter of about 3 mm to about 9 mm. In other embodiments, the diameter is about 3 mm to
- 30 about 8 mm, about 3 mm to about 7 mm, about 3 mm to about 6 mm, about 3 mm to about 5 mm, about 3 mm to about 4 mm, about 4 mm to about 9 mm, about 4 mm to about 8 mm, about 4 mm to about 7 mm, about 4 mm to about 6 mm, or about 4 mm to about 5 mm. In some embodiments, the diameter is about 4 mm.

- 35 In some embodiments, the at least one membrane filter is characterized by a thickness

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of about 150  $\mu\text{m}$  to about 1000  $\mu\text{m}$ . In other embodiments, the thickness is about 150  $\mu\text{m}$  to about 900  $\mu\text{m}$ , about 150  $\mu\text{m}$  to about 800  $\mu\text{m}$ , about 150  $\mu\text{m}$  to about 700  $\mu\text{m}$ , about 150  $\mu\text{m}$  to about 600  $\mu\text{m}$ , about 150  $\mu\text{m}$  to about 400  $\mu\text{m}$ , about 200  $\mu\text{m}$  to about 1000  $\mu\text{m}$ , about 200  $\mu\text{m}$  to about 900  $\mu\text{m}$ , about 200  $\mu\text{m}$  to about 800  $\mu\text{m}$ , about 200  $\mu\text{m}$  to about 700  $\mu\text{m}$ , about 200  $\mu\text{m}$  to about 600  $\mu\text{m}$ , about 200  $\mu\text{m}$  to about 500  $\mu\text{m}$ , or about 200  $\mu\text{m}$  to about 400  $\mu\text{m}$ . In some embodiments, the thickness is about 200  $\mu\text{m}$  to about 400  $\mu\text{m}$ .

In some embodiments, the device is capable of filtering particles with a particle size of more than 0.1  $\mu\text{m}$ . In other embodiments, the device is capable of filtering particles with a particle size of more than 0.2  $\mu\text{m}$ , 0.3  $\mu\text{m}$ , 0.4  $\mu\text{m}$ , 0.5  $\mu\text{m}$ , 0.6  $\mu\text{m}$ , 0.7  $\mu\text{m}$ , 0.8  $\mu\text{m}$ , 0.9  $\mu\text{m}$ , or 1  $\mu\text{m}$ .

In some embodiments, the device is characterized by a particulate filtration efficiency at 0.1  $\mu\text{m}$  of more than about 90%. In other embodiments, the particulate filtration efficiency is more than about 91%, about 92%, about 93%, about 94%, about 95% or about 96%.

The present disclosure also concerns a use of the membrane filter device in conjunction with a capnography system. The membrane filter device may be attached to a side stream of monitoring line of the capnography system. The membrane filter device may be a single use device, thus may be disposed after use.

The device may be attached to a capnography system by connecting tubings to each of the protrusions, where the tubings are connected to the capnography system. This allows for the air to flow from the patient to the capnography system for  $\text{CO}_2$  monitoring. Bacteria and viruses may be filtered via the membrane filter device and hence, may not enter the capnography system from the patient. The device can then be detached from the capnography system after use and disposed, reducing the potential for cross-contamination between patients when the capnography system is used for different patients.

#### Examples

##### Cashew testa extract

- Appearance: Brown liquid

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- Physicochemical characteristics: High radical scavenging activity (antioxidant activity)
- Composition: Mixture of compounds including catechin, epicatechin, tannic acid
- Thermally stable up 200 °C
- 5 • Solubility in water is 2850 g/L

#### Extraction of cashew testa – Comparator

- The skins were milled at 500 rpm for 1 h until it turned into a fine powder, using a ball miller. Then, the powder was collected and MilliQ water in a ratio of 1:10 was added.
- 10 The entire mixture was stirred and incubated in a water bathe for 1 h, at 37 °C. Next, it was centrifuged at 10,000g for 10 min at 4 °C. The supernatant was taken, carefully passed through a 0.2-µm filter, and freeze dried.

#### Extraction of cashew testa – Method 1

- 15 To obtain the cashew testa extract, the method as disclosed in International Journal of Engineering Technology Science and Research, Vol 4(8), page 671-675 (ISSN: 2394-3386) can be used. For example, the testa can be boiled in water at 40 °C to 100 °C for 1-24 hours. This extracts the water soluble polyphenols from the testa such as tannins. After heating, the solution is carefully filtered to remove any remaining solid mass.

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#### Example 1: Synthesis of iron-polyphenol complex particles (Fe-cashew core covered with a shell of $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ) - Composite A

- Fresh cashew extract was prepared as previously described and allowed to cool to room temperature. 1-50 mL Cashew extract and 2-10 g of iron powder was combined and
- 25 this mixture was bubbled with nitrogen gas for 1 h. During this process, iron and cashew extract would be linked to form the compound Fe-cashew. Separately, the solution of 1-90 mL 0.1 M  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  was prepared, and bubbled with nitrogen gas for 1 h. Subsequently, the Fe-cashew solution and 0.5 M  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  were combined. The reaction was continued for 24 h under nitrogen, and the product was stored at 4 °C.
- 30 This would produce a Fe-cashew as the core of the nanoparticles, which is covered with a shell of  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ .

- Alternatively, 2-10 g Fe + 1-50 ml of cashew extract were combined and incubated at 1 h, room temperature with constant agitation. Then 1-90 ml of 0.1M  $\text{FeCl}_3$  was added
- 35 and the mixture was incubated at 1 h, room temperature with constant agitation. The

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entire mixture was centrifuged at 5000 rpm and the precipitate were collected. It was washed with water and then ethanol.

In the examples as disclosed herein, different types of Fe salts were tested; for example,

5 FeCl<sub>3</sub>, FeSO<sub>4</sub>, Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>, Fe(NO<sub>3</sub>)<sub>3</sub>, Fe(NO<sub>3</sub>)<sub>2</sub>.

Example 2: Synthesis of iron-polyphenol complex particles (Fe core covered with a shell of cashew-Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O) - Composite B

2-10 g of iron powder in water was bubbled with nitrogen gas for 1 h. Separately, 1-50  
10 ml cashew solution and 1-90 ml 0.1 M Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O was combined, and bubbled with nitrogen gas for 1 h to form a cashew-Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O linked compound. Subsequently, the iron solution and cashew-Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O solution were combined. The reaction was continued for 24 h under nitrogen, and the product was stored at 4 °C. This would produce nanoparticle with Fe as the core, which is covered with a shell of cashew-  
15 Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O.

Example 3: Synthesis of cashew extract activated iron powder (composite C)

Fresh iron powder (1-10 g) was mixed with cashew testa extract (1-50 ml), and the mixture was stirred at 80 °C for 24 hours. After cooling to room temperature, solid  
20 residuals of the composite C were collected.

Example 4: Synthesis of cashew extract iron nanoparticles (composite D)

A solution of 0.1 M FeCl<sub>3</sub> was prepared by adding 16.23 g of FeCl<sub>3</sub> in 1 litre of Milli-Q water. Subsequently, 1-90 mL 0.1 M FeCl<sub>3</sub> solution was added to 1-50 mL cashew  
25 extract. The formation of iron-cashew nanoparticles was marked by the appearance of precipitate, and this was collected by centrifuging at 7000 rpm. Then the iron-cashew nanoparticles powders were frozen at -20 °C and then were dried in a freeze-dryer at -45 °C with the pressure at 10 Pa for 24 h.

30 Alternatively, 1-90 mL 0.1 M FeCl<sub>3</sub> solution and 1-50 mL cashew extract can be incubated at 1 h, room temperature. Then the entire mixture was centrifuged at 5000 rpm and the precipitate were collected. It was washed with water and then ethanol.

Example 5: Synthesis of cashew extract iron nanoparticles (composite E)

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1-90 mL 0.1 M  $\text{FeCl}_3$  + 1-50 mL cashew testa extract were combined and incubated at 1 h, room temperature. Then 1 M NaOH was added until the pH was 11. The entire mixture was centrifuged at 5000 rpm and the precipitate were collected. It was washed with water and then ethanol.

5

Example 6: Fe-  $\text{FeCl}_3$  as the core and cashew as the shell (composite F)

1-10 g Fe + 1-90 ml of 0.1M  $\text{FeCl}_3$  were combined and incubated at 1 h, room temperature with constant agitation. Then 1-50ml of cashew extract was added and the mixture was incubated at 1 h, room temperature with constant agitation. The entire mixture was centrifuged at 5000 rpm and the precipitate were collected. It was washed with water and then ethanol.

10

Energy-dispersive X-ray Spectroscopy (EDX) analysis of iron particles and/or iron oxide particles

15 The EDX results as shown below are based on Example 1, 2 and 6, using different amounts of iron particle, iron oxide precursor and cashew testa extract.

| Element | Wt%    | Wt% Sigma |
|---------|--------|-----------|
| C       | 50.60  | 0.62      |
| O       | 27.13  | 0.56      |
| Cl      | 0.89   | 0.08      |
| Fe      | 21.37  | 0.42      |
| Total:  | 100.00 |           |

| Element | Wt%    | Wt% Sigma |
|---------|--------|-----------|
| C       | 36.22  | 0.36      |
| O       | 39.39  | 0.32      |
| Cl      | 1.14   | 0.04      |
| Fe      | 23.25  | 0.23      |
| Total:  | 100.00 |           |

| Element | Wt%    | Wt% Sigma |
|---------|--------|-----------|
| C       | 31.15  | 0.40      |
| O       | 40.18  | 0.34      |
| Cl      | 1.43   | 0.05      |
| Fe      | 27.24  | 0.27      |
| Total:  | 100.00 |           |

| Element | Wt%   | Wt% Sigma |
|---------|-------|-----------|
| C       | 31.25 | 0.38      |
| O       | 40.38 | 0.33      |

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|        |        |      |
|--------|--------|------|
| Cl     | 0.77   | 0.04 |
| Fe     | 27.61  | 0.26 |
| Total: | 100.00 |      |

| Element | Wt%    | Wt% Sigma |
|---------|--------|-----------|
| C       | 4.52   | 0.31      |
| O       | 15.09  | 0.18      |
| Al      | 0.28   | 0.05      |
| Si      | 0.38   | 0.05      |
| Cl      | 0.44   | 0.04      |
| Fe      | 79.29  | 0.32      |
| Total:  | 100.00 |           |

#### Scanning Electron Microscope (SEM) analysis

SEM results from Example 1, 2 and 6 shows a particle size distribution from about 1  $\mu\text{m}$  to about 10  $\mu\text{m}$ .

#### Levels of $\cdot\text{OH}$ generated from iron-iron-oxide composition in water analyzed by hydroxyphenyl fluorescein (HPF) probe

Example 1 using  $\text{Fe}(\text{NO}_3)_3$  and Example 4 using  $\text{FeCl}_3$  were used as the samples.

0.01 g of sample was added into 1.5 mL centrifuge tubes. 1 mL of 10  $\mu\text{M}$  HPF testing solution was added in each sample. The solution was well mixed by vortex and kept at room temperature in the dark. At a certain time point, the solution was centrifuged (16800 rpm  $\times$  5 min), and 100  $\mu\text{L}$  of solution was transferred to a black 96 well microplate for fluorescence testing. Fluorescence at 490/ 515 nm was collected with a microplate reader.

The type of ROS that  $\text{FeNO}_3$ -Cashew (Example 1) releases in the dark after 2 hours was  $\cdot\text{OH}$  radicals.  $\text{FeCl}_3$ -cashew (using Method 1) does not release  $\cdot\text{OH}$  radicals (Figure 18).

#### Levels of $\cdot\text{O}_2$ radical from iron-cashew particles measured by Nitroblue Tetrazolium (NBT)

Example 1 using  $\text{Fe}(\text{NO}_3)_3$  and Example 4 using  $\text{FeCl}_3$  were used as the samples.

0.2 g of iron-cashew particles was added to 10 mL of 1000  $\text{mgL}^{-1}$  NBT in water and kept in the dark. At a certain time point, the absorption spectrum of NBT was measured by a UV-vis-NIR spectrophotometer. The absorption peak NBT continues to decrease, at 2 h, indicating the continuous generation of  $\cdot\text{O}_2$  radicals by  $\text{FeCl}_3$ -cashew and react with NBT.

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The comparator was prepared in a similar manner. At the 2 h interval, there is no change in the absorbance value of NBT.

5 Anti-microbial effect of iron-iron oxide composition against *S. aureus*

|                  | 5 min                                           | 15 min                                             | 1 hour                                             | 24 hour                      |
|------------------|-------------------------------------------------|----------------------------------------------------|----------------------------------------------------|------------------------------|
| <b>Untreated</b> | 4.64 x 10 <sup>7</sup> CFU/ml                   | 4.0 x 10 <sup>7</sup> CFU/ml                       | 1.44 x 10 <sup>7</sup> CFU/ml                      | 6.8 x 10 <sup>6</sup> CFU/ml |
| <b>Treated</b>   | 6 x 10 <sup>4</sup> CFU/ml<br>(3 log reduction) | 0 x 10 <sup>4</sup> CFU/ml<br>( > 3 log reduction) | 0 x 10 <sup>2</sup> CFU/ml<br>( > 5 log reduction) | 0<br>( > 6 log reduction)    |

Quantitative Suspension Test for Evaluation of Bacterial Activity

| Test Organism                               | Initial suspension (N)<br>No=(1/10N)<br>1.5x10 <sup>8</sup> ≤ N ≤ 5x10 <sup>8</sup> | Final Count Na        |                       | R (Log10 Reduction) =<br>Log No - Log Na<br>Criteria: R ≥ 5.0 |       |
|---------------------------------------------|-------------------------------------------------------------------------------------|-----------------------|-----------------------|---------------------------------------------------------------|-------|
|                                             |                                                                                     | 1 min                 | 5 min                 | 1 min                                                         | 5 min |
| <i>Staphylococcus aureus</i><br>ATCC 6538   | 2.0 x 10 <sup>8</sup>                                                               | 3.0 x 10 <sup>2</sup> | 1.0 x 10 <sup>2</sup> | 5.82                                                          | 6.30  |
| <i>Escherichia coli</i><br>ATCC 10536       | 1.8 x 10 <sup>8</sup>                                                               | 90                    | 10                    | 6.31                                                          | 7.26  |
| <i>Pseudomonas aeruginosa</i><br>ATCC 15442 | 1.5 x 10 <sup>8</sup>                                                               | 1.0 x 10 <sup>3</sup> | 1.0 x 10 <sup>2</sup> | 5.20                                                          | 6.20  |
| <i>Enterococcus faecium</i><br>ATCC 6057    | 3.0 x 10 <sup>8</sup>                                                               | 2.2 x 10 <sup>3</sup> | 6.0 x 10 <sup>2</sup> | 5.14                                                          | 5.70  |
| <i>Salmonella typhimurium</i><br>ATCC 13311 | 2.7 x 10 <sup>8</sup>                                                               | 5.0 x 10 <sup>2</sup> | 80                    | 5.73                                                          | 6.53  |

The requirements of bactericidal efficacy according to EN 1276 was satisfied.

10

Anti-viral efficacy

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| Virus Types                                  | (NO) | lg(P <sub>0a</sub> ) (lgTCID <sub>50</sub> /mL) | lg(P <sub>5a</sub> ) (lgTCID <sub>50</sub> /mL) | lg(P <sub>5a</sub> ) (lgTCID <sub>50</sub> /mL) |
|----------------------------------------------|------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| SARS-CoV-2<br>MDCK                           | 1    | 6.67                                            | 6.53                                            | 4.32                                            |
|                                              | 2    | 6.63                                            | 6.51                                            | 4.35                                            |
|                                              | 3    | 6.59                                            | 6.48                                            | 4.27                                            |
| Average Value of<br>lgTCID <sub>50</sub> /mL |      | 6.63                                            | 6.51                                            | 4.31                                            |
| Antiviral Activity Value                     |      | 2.19                                            |                                                 |                                                 |
| Antiviral Activity Rate (%)                  |      | 99.36                                           |                                                 |                                                 |

With SARS-CoV-2 and based on ISO 18184:2019, the iron-iron oxide composition showed an antiviral activity rate of 99.36% after a 2 h contact time.

#### 5 Process of making the membrane filter (Method A)

The iron-iron oxide composition is applied to the fabric/filter by a mangle at the entry to a stenter. The fabric/filter is then cured at 120 °C for 7 min through a 10 chamber stenter. It is then pass through the stenter again by applying a layer of water repellent coating to enhance the adhesion of the metal oxide to the fabric. Using this process, a coating is created on the surface on the fabric. It also assists to optimize the Particle filtration efficiency of the mask.

#### Anti-bacterial test based on Textile Materials Assessment (AATCC 100-2012)

The material was tested using the following conditions:

- 15 • Staphylococcus aureus ATCC 29213
- Dilution medium: PBS
- Sample size # layers: 1
- Sterilization: None
- Neutralizer: 0.9% NaCl + 0.2% Tween
- 20 • Target inoc. Level: (1.0-2.0) x 10<sup>6</sup> CFU/ml
- Inoculum size: 1.0 ml
- Contact time: 24 h
- Temperature: 37 +/- 2 °C

It was found that after 24 h, 98-100% reduction on bacterial was observed.

25

#### Bacterial Filtration Efficiency 1 (ASTM TEST METHOD F2101)

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A suspension of *Staphylococcus aureus*, ATCC 29213, was delivered to the test article to determine filtration efficiency. A challenge level of greater than  $10^7$  colony forming units (CFU) was pumped through an aerosol spray. This method was adapted from ASTM F2101. This test was not performed at a fixed flow rate of 28.3 L/min, but at a more strict level. The aerosol particles were not drawn through are drawn through a six stage Andersen sampler for collection. However, adequate controls are included to verify the reliability of this study. All test method acceptance criteria were met. The conditions are as follows:

Challenge Flow Rate: Aerosol spray

- 10 Conditioning Parameters:  $85 \pm 5\%$  relative humidity (RH) and  $21 \pm 5^\circ\text{C}$  for a minimum of 4 hours

Area Tested: 15 cm x 15 cm

Side Tested: Black side

Challenge Level :  $1.8 \times 10^7$  CFU

- 15 Mean Particle Size (MPS):  $\sim 0.8 \mu\text{m}$

The 5 samples tested has an average bacterial filtration efficiency of 97.2%. As the membrane filter can filter the *S. aureus* ATCC 29213 bacteria which has a size of  $\sim 0.8 \mu\text{m}$  by up to 99.9998%, the face mask meets the fine particulate matter of 2.5 (PM<sub>2.5</sub>).

20

Bacterial Filtration Efficiency 2 (BS EN 14683:2019)

Area contacting with the bacterial challenge: Inside of the mask

Flowrate:  $28.3 \pm 0.3$  L/min

Mean particle size of the challenge aerosol:  $3 \mu\text{m} \pm 0.3 \mu\text{m}$

- 25 Test area: Approximately 50 cm<sup>2</sup>

The 5 face masks tested all have a Bacterial Filtration Efficiency of more than 99.85%.

Anti-viral efficacy

- 30 Cell lines: Madin-Darby Canine Kidney (MDCK) cell

Viruses: Influenza A virus (PR8 #2-3)

Drug/Chemical: Cover-slides coated with anti-microbial agent (Labelled Control sample, sample 28 and sample 60)

Media: DMEM-E10, Overlay media

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*Virus treatment with coverslides coated with anti-microbial agent for 1h incubation* – Influenza A virus were diluted to  $10^5$  PFU/ 100  $\mu$ L and added upon the control, sample 1 and sample 2 coated cover-slides. After which, a thin parafilm was used to cover on each slide to ensure virus contact with the coated cover-slide. Supernatants were  
5 harvested for plaque assay at 1 h post-incubation.

*Plaque assay* – MDCK cells were inoculated on 12-well plates separately. Influenza-treated supernatants were serially diluted to  $10^{-4}$  and 250  $\mu$ L of diluted supernatants were added to MDCK cells. Plates were incubated for 1 h with 15 mins rocking intervals.  
10 Plates were then washed with 1x PBS twice and 0.3% agarose overlay media were added to each well. Plates were incubated 2 days. Lastly, overlay media was removed and crystal violet was added to stain for countable plaques. Plaques were then calculated using plaque forming units per mL.

15 In comparison to control group, the result showed that the samples was sufficient to inhibit virus > 4.5-log unit (or at least 0.5-log unit inhibition). The average of plaque assay virus titres found from control and samples were  $4.53 \times 10^4$  PFU/mL and 0 PFU/ml (or at least  $9.06 \times 10^3$  PFU/mL) respectively.

20 With P22 bacteriophage and based on ISO 18184:2019, the membrane filter showed an overall reduction of 99.98% after a 24 h contact time.

With Enterovirus 71 and based on ISO 18184:2014, the membrane filter showed an overall reduction (antiviral activity rate) of 99.92 % after a 2 h contact time.

25

With H1N1 Influenza A virus and based on ISO 18184:2014, the membrane filter showed an overall reduction (antiviral activity rate) of 99.93 % after a 2 h contact time.

30 With H3N2 Influenza A virus and based on ISO 18184:2014, the membrane filter showed an overall reduction (antiviral activity rate) of 97.25 % after a 5 min contact time. The membrane filter showed an overall reduction (antiviral activity rate) of 99.52 % after a 2 h contact time. In contrast, membrane filter with iron oxide alone showed an overall reduction (antiviral activity rate) of 99.12 % after a 2 h contact time, while membrane filter with cashew testa extract alone showed an overall reduction (antiviral  
35 activity rate) of 92.43 % after a 2 h contact time.

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With SARS-CoV-2 virus and based on ISO 18184:2019, the membrane filter showed an overall reduction (antiviral activity rate) of 99.08 % after a 2 h contact time.

5 Cytotoxicity Test (ISO 10993-5; ISO 10993-12)

The membrane filter has a Grade 0, indicating that it has no cytotoxic effect.

Process of making the membrane filter (Method B)

- Pad-Dry-Cure technique is used. The Cashew testa extract was treated with 1-80  
10 percent on weight of fabric/filter (owf) under room temperature. The treated fabric/filter  
was passed between the rollers two to three times at a uniform pressure at 1.5 bar for  
better penetration of finishing agent and to squeeze out excess liquid from the fabric.  
The fabric/filter was then dried at 110°C and cured at 130°C in a curing chamber within  
3 minutes. The cashew testa extract has a 99% antimicrobial activity against the  
15 common gram positive bacteria *Staphylococcus aureus*.

Anti-microbial test

- This test is performed with treated and non-treated samples of the test material. The  
material was cut into 4.8 cm diameter. In accordance with AATCC 100 test procedure,  
20 1 ml of test organism suspension at about  $1-2 \times 10^5$  CFU/ml is inoculated to the test  
sample. Samples of the cashew testa extract composition and iron particles and/or iron  
oxide particles were tested. The inoculated swatches were incubated for a specified  
contact time. At the appropriate contact time, neutralizing broth was added to each  
container and the containers were shaken for 1 minute to release the inoculum from the  
25 test swatches and into the neutralizing broth. Serial dilutions were made and the plates  
incubated. After incubation, colonies of recovered are counted and used to determine  
percent reductions.

- The results below relates to fabric samples. Percent reductions are determined by  
30 comparing the sample after the contact time to the sample immediately after  
inoculation. Percent reduction is translated into log reduction by the following:

90% reduction = 1 log reduction;

i.e. 1,000,000 reduced to 100,000 is a 1 log reduction

99% reduction = 2 log reduction;

- 35 i.e. 1,000,000 reduced to 10,000 is a 2 log reduction

99.9% reduction = 3 log reduction;  
i.e. 1,000,000 reduced to 1,000 is a 3 log reduction  
99.99% reduction = 4 log reduction;  
i.e. 1,000,000 reduced to 100 is a 4 log reduction

- 5
- Testing information:
- *Staphylococcus aureus* ATCC 29213
  - Dilution medium: PBS
  - Sample size # layers: 1
  - Sterilization: None
- 10
- Neutralizer: 0.9% NaCl + 0.2% Tween
  - Target inoc. Level: (1.0-2.0) x 10<sup>5</sup> CFU/ml
  - Inoculum size: 1.0 ml +/- 0.1 ml
  - Contact time: 24 h

Temperature: 37 +/- 2° C

| AATCC 100 Test Method at 24 hours contact time | <i>S. aureus</i> |
|------------------------------------------------|------------------|
| Iron-Iron oxide composition                    | 2 log reduction  |
| Iron-Iron oxide composition on filter          | 2 log reduction  |

- 15
- Results for test against H3N2 Virus

| Test Results against H3N2 at 2 hour incubation | Iron-Iron oxide composition  | Iron-Iron oxide composition on filter |
|------------------------------------------------|------------------------------|---------------------------------------|
| Results                                        | 99.52%<br>2.32 log reduction | 99.52%<br>2.32 log reduction          |

Results for test against HCoV-229E Virus

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| Virus Types                                         | NO | $\lg(V_{\text{before}})$<br>( $\lg\text{TCID}_{50}/\text{mL}$ ) | $\lg(V_{\text{before}})$<br>( $\lg\text{TCID}_{50}/\text{mL}$ ) | $\lg(V_{\text{before}})$<br>( $\lg\text{TCID}_{50}/\text{mL}$ ) |
|-----------------------------------------------------|----|-----------------------------------------------------------------|-----------------------------------------------------------------|-----------------------------------------------------------------|
| HCoV-229E<br>MDCK cells                             | 1  | 6.32                                                            | 6.28                                                            | 4.44                                                            |
|                                                     | 2  | 6.37                                                            | 6.34                                                            | 4.36                                                            |
|                                                     | 3  | 6.34                                                            | 6.21                                                            | 4.47                                                            |
| Average Value of<br>$\lg\text{TCID}_{50}/\text{mL}$ |    | 6.34                                                            | 6.28                                                            | 4.46                                                            |
| Antiviral Activity Value                            |    | 1.88                                                            |                                                                 |                                                                 |
| Antiviral Activity Rate (%)                         |    | 98.68                                                           |                                                                 |                                                                 |
| Virus Types                                         | NO | $\lg(V_{\text{before}})$ ( $\lg\text{TCID}_{50}/\text{mL}$ )    | $\lg(V_{\text{before}})$ ( $\lg\text{TCID}_{50}/\text{mL}$ )    | $\lg(V_{\text{before}})$ ( $\lg\text{TCID}_{50}/\text{mL}$ )    |
| HCoV-229E<br>MDCK cells                             | 1  | 6.39                                                            | 6.35                                                            | 4.39                                                            |
|                                                     | 2  | 6.45                                                            | 6.42                                                            | 4.37                                                            |
|                                                     | 3  | 6.42                                                            | 6.39                                                            | 4.36                                                            |
| Average Value of<br>$\lg\text{TCID}_{50}/\text{mL}$ |    | 6.42                                                            | 6.39                                                            | 4.37                                                            |
| Antiviral Activity Value                            |    | 2.65                                                            |                                                                 |                                                                 |
| Antiviral Activity Rate (%)                         |    | 99.10                                                           |                                                                 |                                                                 |
| Virus Types                                         | NO | $\lg(V_{\text{before}})$ ( $\lg\text{TCID}_{50}/\text{mL}$ )    | $\lg(V_{\text{before}})$ ( $\lg\text{TCID}_{50}/\text{mL}$ )    | $\lg(V_{\text{before}})$ ( $\lg\text{TCID}_{50}/\text{mL}$ )    |
| HCoV-229E<br>MDCK cells                             | 1  | 6.42                                                            | 6.39                                                            | 4.37                                                            |
|                                                     | 2  | 6.46                                                            | 6.43                                                            | 4.36                                                            |
|                                                     | 3  | 6.44                                                            | 6.41                                                            | 4.34                                                            |
| Average Value of<br>$\lg\text{TCID}_{50}/\text{mL}$ |    | 6.44                                                            | 6.41                                                            | 4.36                                                            |
| Antiviral Activity Value                            |    | 2.08                                                            |                                                                 |                                                                 |
| Antiviral Activity Rate (%)                         |    | 99.17                                                           |                                                                 |                                                                 |

Membrane filter device - Sub-micron Particulate Filtration Efficiency (PFE) at 0.1  $\mu\text{m}$

ASTM F2299/ F2299M - 03 (Reapproved 2017) - Standard Test Method for Determining

- 5 the Initial Efficiency of Materials Used in Medical Face Masks to Penetration by Particulates Using Latex Spheres.

Unneutralized aerosol which represents a more natural state, is used in this test procedure, referring to FDA guidance document on surgical masks (FDA-2003-D-0305).

- After airflow and aerosol stability is established, sample and record upstream and  
10 downstream aerosol counts for a minimum 5 counts at each position, using a -minute sampling time.

Average the upstream counts and the downstream counts, then calculate the efficiency by the following definition:

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Efficiency (%) =  $[1 - (\text{average downstream counts} / \text{average upstream counts})] \times 100$

Test conditions of samples:

Flow rate: 28.3 L/min

Face velocity : 5.1 cm/s

5 Exposed Specimen Area: 91.6 cm<sup>2</sup>

Relative Humidity of Test Airflow: 35 ± 5 %

Temperature of Test Airflow: 22 ± 2 °C

| Material | Particle Diameter (µm) | Particle Diameter Standard Deviation (µm) | Pressure Drop (kPa) | Efficiency (PFE) % | Average efficiency % |
|----------|------------------------|-------------------------------------------|---------------------|--------------------|----------------------|
| 1        | 0.1                    | ± 0.01                                    | 0.068               | 96.3               | 96.6                 |
| 2        | 0.1                    | ± 0.01                                    | 0.075               | 97.4               |                      |
| 3        | 0.1                    | ± 0.01                                    | 0.067               | 95.8               |                      |
| 4        | 0.1                    | ± 0.01                                    | 0.072               | 97.1               |                      |
| 5        | 0.1                    | ± 0.01                                    | 0.075               | 96.5               |                      |

10 Membrane filter device - Microbial Cleanliness (Bioburden) (BS EN 14683:2019 / ISO 11737-1:2018)

Number of Tested Specimens: 5

Extraction Liquid: Peptone 1g/l, NaCl 5g/l and Tween 20 2g/l

Extraction Volume: 300 mL

15 Extraction Time: 5 min

Filtration Volume: 100 mL

Culture Media: TSA (Tryptic Soy Agar)

SDA (Sabouraud Dextrose Agar with Chloramphenicol)

Incubation Condition: 3 days at 30°C (TSA)

20 7 days at 20 - 25°C (SDA)

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| Specimen | Specimen Weight (g) | CFU / Specimen          |             | Microbial Cleanliness |       |
|----------|---------------------|-------------------------|-------------|-----------------------|-------|
|          |                     | Aerobic microbial Count | Fungi Count | Total CFU / Specimen  | CFU/g |
| 1        | 3.71                | 42                      | 1           | 43                    | 12    |
| 2        | 3.68                | 28                      | 0           | 28                    | 8     |
| 3        | 3.47                | 23                      | 0           | 23                    | 7     |
| 4        | 3.61                | 49                      | 1           | 49                    | 14    |
| 5        | 3.69                | 45                      | 0           | 45                    | 12    |

#### Evaluation of filter device in operating theatre

The filter devices were used in operating theatres at a Singapore hospital. The operating theatres were equipped with GE Aisys CS2 or GE Avance CS2 anaesthesia machines.

#### Testing protocol

Test 1 involves connecting a partially air-filled 20 cc plastic syringe to the D-fend water trap of the airway module (part of the capnography system) in a checked switch-on anaesthesia machine.

Test 2 involves repeating Test 1 but with the filter device attached firmly between the partially air-filled 20 cc plastic syringe and the D-fend water trap of the airway module. In the presence of patency and absence of structural leakage of the viral filter, the airway module will suck and empty the partially air-filled syringe within 5 sec showing the negative pressure sampling function of the capnography system is working normally. The filter device is then considered to pass test 2.

Test 3 involves connecting the end-tidal CO<sub>2</sub> sampling line to the capnography system and sample one exhaled breath from the patient through the surgical face mask. A single positive capnograph waveform shows the CO<sub>2</sub> detection of the capnography system is working normally.

Test 4 involves repeating Test 3 but with the filter device attached at the patient end of the sampling line. The filter device is considered to pass test 4 when the exhaled breath from the patient is detected as a single positive capnograph waveform.

Test 5 involves placing the sampling line with the filter device attached close to the nasal region of patient's external surgical mask to detect and monitor the capnograph waveforms. The filter device is considered to pass test 5 when patient's breathing

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pattern is captured entirely with the displayed capnograph waveform throughout the surgical procedure.

#### Results

5 15 patients of both genders, races and aged between 30-80 consented to participate in the study. The capnography system with the filter device was monitored for about 10-50 min. All filter devices passed test 2, 4 and 5. The results were consistently positive with no failure. Patient's demography and surgical condition have no impact on the positive testing of the filter devices.

10

It will be appreciated that many further modifications and permutations of various aspects of the described embodiments are possible. Accordingly, the described aspects are intended to embrace all such alterations, modifications, and variations that fall within the spirit and scope of the appended claims.

15

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

20

Throughout this specification and the claims which follow, unless the context requires otherwise, the phrase "consisting essentially of", and variations such as "consists essentially of" will be understood to indicate that the recited element(s) is/are essential i.e. necessary elements of the invention. The phrase allows for the presence of other  
25 non-recited elements which do not materially affect the characteristics of the invention but excludes additional unspecified elements which would affect the basic and novel characteristics of the method defined.

30

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

### Claims

1. A capnography filter device, comprising:
  - a) an elongated housing having a first end and a second end, the first and second ends each independently comprising a protrusion;
  - b) at least one filter housed within the housing;wherein the at least one filter is characterized by a pore size of about 0.2  $\mu\text{m}$  to about 0.5  $\mu\text{m}$ .
2. The capnography filter device according to claim 1, wherein the at least one filter is in contact with an inner side of the housing at the first end or second end.
3. The capnography filter device according to claim 1 or 2, wherein the housing is characterized by an inner diameter configured to be substantially similar to an inner diameter of an attachable tubing when in use.
4. The capnography filter device according to any one of claims 1 to 3, wherein the housing is separable in order for an interior space of the housing to be accessed.
5. The capnography filter device according to any one of claims 1 to 4, wherein the housing further comprises connecting means.
6. The capnography filter device according to any one of claims 1 to 5, wherein the capnography filter device further comprises an insert with a through hole.
7. The capnography filter device according to claim 6, wherein the insert is configured to frictionally fit inside an interior space of the housing and configured to engage the at least one filter.
8. The capnography filter device according to claim 6 or 7, wherein the insert is a Teflon insert.
9. The capnography filter device according to any one of claims 1 to 8, wherein the housing is characterized by an outer diameter of about 5 mm to about 15 mm.

10. The capnography filter device according to any one of claims 1 to 9, wherein the housing is characterized by an outer diameter of about 9 mm.
11. The capnography filter device according to any one of claims 1 to 10, wherein  
5 the housing is characterized by an inner diameter of about 2.5 mm to about 4.5 mm.
12. The capnography filter device according to any one of claims 1 to 11, wherein the housing is characterized by an inner diameter of about 4 mm.
- 10 13. The capnography filter device according to any one of claims 1 to 12, wherein the housing is characterized by a length of about 15 mm to 30 mm.
14. The capnography filter device according to any one of claims 1 to 13, wherein the housing is characterized by a length of about 22 mm.
- 15 15. The capnography filter device according to any one of claims 1 to 14, wherein the housing is characterized by an inner capacity of about 60 mm<sup>3</sup> to about 1000 mm<sup>3</sup>.
16. The capnography filter device according to any one of claims 1 to 15, wherein  
20 the at least one filter is two filters.
17. The capnography filter device according to claim 16, wherein the two filters are spaced apart.
- 25 18. The capnography filter device according to claim 15 or 16, wherein the two filters are each independently in contact with respective inner sides of the first end and second end.
19. The capnography filter device according to any one of claims 1 to 18, wherein  
30 the at least one filter is a membrane filter, HEPA filter or N95 filter.
20. The capnography filter device according to any one of claims 1 to 19, wherein the at least one filter comprises an anti-microbial composition, an anti-viral composition, or a combination thereof.
- 35

21. The capnography filter device according to claim 20, wherein the anti-microbial composition comprises:
- a) a cashew testa extract; and
  - b) iron-iron oxide core-shell particles, the core is an elemental iron core or an iron alloy core, and the shell is an iron oxide shell;
- 5 wherein the cashew testa extract comprises a component selected from amino acid, protein, carbohydrate, phenolic compounds or a combination thereof; and wherein the iron-iron oxide core-shell particles are at least partially passivated by a component of the cashew testa extract.
- 10
22. The capnography filter device according to claim 21, wherein the phenolic compounds are selected from tannins, catechin, epicatechin, epigallocatechin, p-coumaric, gallic acid, or a combination thereof.
- 15
23. The capnography filter device according to claim 21 or 22, wherein the iron-iron oxide core-shell particles is characterized by a particle size of about 1  $\mu\text{m}$  to about 100  $\mu\text{m}$ .
24. The device according to any one of claims 21 to 23, wherein the iron-iron oxide composition relative to the at least one filter is about 0.1% w/w to about 5% w/w.
- 20
25. The capnography filter device according to any one of claims 21 to 24, wherein the shell has a thickness of about 50 nm to about 400 nm.
- 25
26. The capnography filter device according to any one of claims 21 to 25, wherein the shell further comprises at least a component of the cashew testa extract.
27. The capnography filter device according to any one of claims 20 to 26, wherein the anti-microbial composition further comprises an excipient selected from a surfactant, a stabilizer, a polymer, drying agent or a combination thereof.
- 30
28. The device according to any one of claims 1 to 27, wherein the capnography filter device is characterized by a particulate filtration efficiency at 0.1  $\mu\text{m}$  of more than about 90%.
- 35

29. The device according to any one of claims 1 to 28, wherein the capnography filter device is characterized by an anti-bacterial activity of at least 2 log reduction.

30. The device according to any one of claims 1 to 29, wherein the capnography  
5 filter device is characterized by an anti-viral activity of at least 2 log reduction.