AirAnswers®

FREQUENTLY ASKED QUESTIONS

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Company:

How important is quality at AirAnswers®?

AirAnswers® takes quality very seriously. We have a well-developed Quality Management System and a quality committee comprised of company-wide experts, who meet monthly to discuss improvements to AirAnswers® quality system. Inspirotec has 5 critical ISO certifications and accreditations for manufacturing excellence (ISO 13485:2016), overall company quality processes and controls (ISO 9001:2015), personnel occupational health and safety management (ISO 45001:2018), best practices for protecting our environment (ISO 14001:2015), and for excellent laboratory practices (ISO/IEC 17025:2017).

How long ago was the company founded?

The company was first commercially launched in 2016, with a focus on airborne allergen detection. In 2018, the company was rebranded and AirAnswers® was born, with a focus on detection of all biological materials down to 0.1 microns in size, including allergens, fungi, viruses, and bacteria.

Device Specifications, Operation & Performance:

What are the dimensions of the AirAnswers® device (AA)?

- 6.91 inches x 4.93 inches
- 175.45 mm x 125.28 mm

What is the weight of the device?

- The device weighs 240 grams (~0.5 lbs)

How is the device powered?

A 15 w power adapter with DC jack is the primary source however, for a limited time period the device can be powered via battery pack.

What does the device collect?

The device collects biological materials in the air (bioaerosols) including allergens, bacteria, fungi, and viruses. At a high flow rate of up to 150 liters of air per minute (LPM), the AirAnswers® device has the ability to capture the smaller, more harmful particles that can get deep into the lungs. AirAnswers® has the capacity to capture airborne fine particles as small as 0.1 microns in size.

How do you know you collect 0.1 micron in size particles?

In recent controlled studies, we have efficiently captured Sars-Cov-2 virus, which has a nominaldiameter of 0.1 microns. In addition, in published studies sampling the domestic microbiome, our device captured numerous airborne bacteria including Staphylococcus, Porphyromonas, Moraxella, Sutterella, and Clostridium (Richardson et al. Microbiome (2019) 7:82). These bacteria range in size from 1-3 microns. Finally, in another controlled study, our device efficiently captured well-defined particles sizes of 0.1, 1.0, and 4.0 microns with equal efficiency.

How does the device work?

The device uses electrokinetic capture to silently circulate air through the device, leaving ultra - fine particles bonded to the steel electrodes on the collection cartridge.

What is the device flow rate?

The device circulates air at a rate up to 150 LPM, allowing for the rapid capture of airborne. Over a 1-day period biological particles from approximately 215,000 liters of air travel through the device and are captured on the steel electrodes of the collection cartridge.

How can you prove the device operates at 150L of air per minute?

From a sample numbering of 179 AAs, across 17 lots manufactured in early 2021, the average flow rate was measured as 155 +/- 30 LPM. For the most accurate representation of total airflow of the device we have measured the flow of each of the over 200 holes on the device.

Each individual hole was measured and validated by 3 separate parties. The flow rate from representative sample units is measured using a commercial grade device: ExTech Hot Wire Anemometer (Extech Model 407123).

How is it totally silent?

It is totally silent because there are no fans, pumps, or other mechanical devices used to move air through the unit. The high voltage at the unit's internal positive electrode generates an electromagnetic field that ionizes air molecules, causing them to flow from the positive electrode toward the negative electrodes (the cartridge electrodes), setting up the airflow that moves through the unit.

How do I operate the device?

Remove the AirAnswers® device from the box, insert a cartridge, and plug-it into a standard outlet. Let it run for the appropriate time for your chosen assessment. Press the red reset button if needed.

What is the purpose of the device reset button?

The device reset button, located on the bottom of device, is used between testing cycles to reset the internal timer. Reset the device after each use. Once the cartridge has been removed, plug the device in, then push and hold the red reset button on the bottom of the device for 10 seconds to reset the internal clock (the light ring will flash during reset). Once this is complete, unplug your device. Your device is now ready to use on your next project.

How do I know the device is working?

If the device is functioning properly, there is a light ring around the bottom of the device that will blink from green to yellow when you plug the device in and then maintain a steady green light after a few seconds and throughout the testing period. If after plugging in the device or pushing the reset button the light ring stays yellow or continues to alternate between yellow and green, check that the cartridge is inserted properly. If the green light goes out during the testing period contact us directly. If performing 1-hour or 1-day sampling, you will have to be mindful of the time and unplug the device after the desired sampling time has elapsed.

How do I insert a new cartridge?

Unsnap the small circular covering on the bottom of the device by pressing the release tab, exposing the cartridge insertion area. Locate one cartridge and gently open the cartridge packaging, exposing the cartridge handle. Grip the handle of the cartridge with your fingers. Do not touch the metal test strips located on the interior of the cartridge. Touching the test strips will compromise the lab testing results. Align the cartridge properly and insert it into the bottom of the device, making sure that all the cartridge fits completely inside, except for the handle. Orientation should match the semi-circular recess in the bottom of the device.

The handle will remain exposed when the cartridge is fully inserted. You should feel a slight snap sensation when properly inserted. Replace the circular covering on the bottom of the device, by snapping it back in place.

How do I remove the cartridge?

Unsnap the small blue circular covering on the bottom of the device by pressing the release tab, exposing the cartridge area. Grip the exposed handle with your fingertips (do not touch the metal test strips, this will compromise the lab test) you will feel a gentle pressure release as you slowly pull the cartridge straight out. In order to protect your cartridge during shipment, place the cartridge back into its original plastic container.

What if I am performing a 5-day run for allergens and I am unable to get to the location in-time to stop the device?

There is a 5-day timer located within the device. Once the device runs for 5-days, it automatically shuts off. The light ring turns to flashing yellow.

What is the lifetime usage of the device?

Because the device has no moving parts, we anticipate reliable use over many years.

How does this device get calibrated in the factory and how often should I calibrate it?

The voltage that controls the flow is set at the factory and checked using QC samples from every lot of device produced. There is no further calibration required in the field.

How is the device self-cleaning?

The device is equipped with an internal cleaning apparatus that cleans the positive electrode upon cartridge insertion.

How is it not contaminated from job site to job site?

Carryover studies have been performed and validated in-house. The bioaerosols that are ionized by the electric field within the device, are attracted and stick only to the negative electrodes (metal strips) in the cartridge. They are removed when the cartridge is removed.

What is the capture efficiency?

In two studies using controlled conditions across defined particle size ranges from 0.1 to 4.0 microns, the average capture efficiency was about 16%

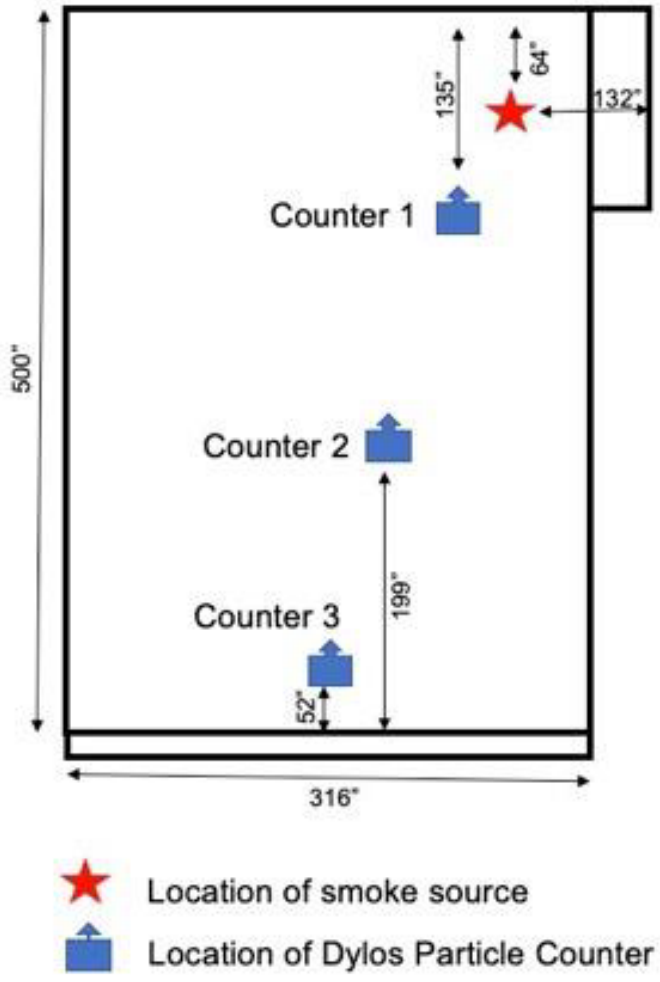
Air Sampling & Device Placement:

Where do I place the device when testing?

If you would like to test a whole indoor environment, we recommend putting the device in a centralized location, 36-48 inches off the floor on a piece of furniture (table, bookcase, nightstand, or desk), near an air recycle point. Leave all interior doors to rooms open. If you would like to test a specific room, place the device in the room and make sure that the door is closed for the entire testing period.

How can one AirAnswers® device location be sufficient to test for bioaerosols in a residential or industrial space?

We performed a study modeling the dynamics of how particles emitted from one point in a space would distribute to other locations across that space. This was done by briefly releasing particulates at one point and following particle counts of PM 0.5 and PM 2.5 size ranges over time at different locations.



Results were from a 2-storey 2500 sq.ft. home and an auditorium that seats 100, both with a standard HVAC system actively moving the air. Particles traversed and filled the spaces with great rapidity; less than 5 minutes. This demonstrated that an AirAnswers® placed in a reasonably central location in a house, or other large space, would capture a representative sample of particles from throughout the entire space.

If air flow is restricted from one space to another by a wall or doors, there may be some air exchange between the spaces, but a second AirAnswers® would be recommended for that space. For open plan office areas with cubicles, one AirAnswers® would suffice.

One device should be placed in any room that is in active use: meeting rooms, food courts, toilets, changing rooms, locker rooms, etc. For a very large space such as an exhibition area, anybioaerosols emitted would be diluted into such a large volume, that the allergen or pathogen would become immeasurably low, equivalent to the open air. We recommend placing AirAnswers® devices at high traffic areas such as registration desks, bathrooms, eating areas, and entrances to the exhibit, since there might be an increased steady state of pathogens or allergens closer to a high density of possible sources.

How long should I run the AirAnswers® device to test a whole indoor space?

The sampling time depends on the test chosen. Each of the tests require the following sampling times:

1-Hour, 24-Hours, 5-Days

- Beta-Glucan
- Mold Genera
- Mycotoxins
- Opportunistic Fungal Pathogen Panel
- Powdery Mildew

3-Days

- SARS-CoV-2

5-Days

- Allergens

How do you determine your sampling time for each test?

Optimal run times were determined through environmental chamber and real-world studies.

For your mold (Beta-Glucan) testing, do I need to run an AirAnswers® device outside as a control?

No, for these main reasons;

AirAnswers Mold (BG) Test Sensitivity: With AirAnswers® mold test, we are detecting living and growing mold in the environment. With spore traps, there is no way to decipher if the collected spores are from viable or nonviable molds. Our mold test measures small fractions of a carbohydrate, known as Beta-glucan or (1→3)-β-Dglucan, that are released into the air by germinating and growing mold. With our device, we are not only capturing spores, but we are also capturing hyphae and free-floating beta- glucan from growing molds. Our test is sensitive enough to detect mold that is growing behind walls and in places where mold is not readily visible. **Due to the sensitivity of the test and because we are measuring growing mold, if the AirAnswers® device is run outside, especially in warmer/humid months and in humid parts of the country, the result will show-up above the range of quantification for our mold (BG) test.**

Establishment of Mold Levels - Spore Trap vs AirAnswers®: Mold levels for spore trap were established based on the belief that the indoor air mold spore level should be corrected for the level of naturally occurring mold spores in the outside environment. There is little or no scientific evidence to support this approach, and some real reasons why it might be inaccurate. In practice, the differential between indoor spore counts and outdoor spore counts shows the efficiency of the HVAC system in excluding exterior populations of spores. The indoor and outdoor spore levels often change at very different rates and can be out of sync with each other. Or, an indoor spore concentration that is lower, or about the same, as that outdoors, might still represent a concerning amount of indoor mold because the indoor spores are more viable than those outdoors. By contrast, AirAnswers® mold levels were developed by collecting hundreds of indoor air samples with the external windows and doors closed. Since our levels were established by testing for Beta Glucan in samples collected from a large number of actual, representative, indoor environments we have levels that better reflect what “Minor”, “Low”, “Moderate”, or “Severe” really means in terms of indoor viable mold (BG) levels. Thus, there is no need for an outside sample. To get an accurate representation of the mold growing in an indoor environment, we recommend closing all external windows and doors prior to running the AirAnswers® device.

What data do you have to prove you’re better than the spore trap?

We have conducted side-by-side studies in actual domestic locations and have a white paper developed in collaboration with Michael Pinto of Wondermakers entitled, “A Comparison of AirAnswers® Sampling to Spore Trap Samples For Mold Investigations.”

The following are highlights from the paper, pertaining to AirAnswers® Capabilities:

- **Ability to capture particulates down to 0.1 micron in size -** Collects smaller particulates that can be the source of health problems

- **Captures and quantifies beta-glucans –** Beta-glucans, located in cell walls of fungi and bacteria are indicative of actively growing mold and can be identified even if the fungal colony has dried out, been disturbed, or been left behind by inadequate remediation

- **Utilizes PCR testing –** Use of sensitive PCR results in detection of fungal spores and small fragments (included in the test results) – these pieces would be missed by a laboratory visually counting only the intact spores in a spore trap sample.

- **A single longer-term sample can be taken as compared to having to collect multiple samples –** Cost effective and significant flexibility to develop a more complete picture of the indoor environment by the indoor air quality professional

- **Large capacity for sampling particulates –** Allows for more accurate, in-depth analysis over an extended length of time of up to 5-days, overcoming the spore trap sample collection time limitation of only minutes.

- **Mold sampling not subject to overloading –** There is no upper limit to the amount of material collected by AirAnswers®. AirAnswers® mold results are not affected by the material overload that degrades and interferes with spore trap collection.

- **Able to detect beta-glucan and specific mold genera in as little as 1-hour –** Useful in determining if a problem exists in structures, where health symptoms are reported, when no visible mold or odors are present

- **No potential for interference in the analysis from capture media or analytical stains –** The particulate is not enmeshed in a suspending compound as is the case with the spore trap

- **A single sample can be used to evaluate multiple contaminants –** In addition to beta-glucans (asan indicator of growing mold) , AirAnswers® can identify specific molds, mycotoxins, and allergens.

What are some of the advantages using AirAnswers® for beta glucan vs. spore trap and traditional microscopy?

- **Viable vs Non-viable Mold:** With AirAnswers® Beta-Glucan test, we are detecting living and growing mold in the environment. With spore traps, there is no way to decipher if the collected spores are from viable or non-viable molds. (1→3)-β-d-glucan levels in the air are known to correlate with fungal mass and severity of symptoms.

- **Variability:** Compared with the variability of spore trap results that change based on multiple areas tested in a particular location, one (1→3)-β-d-glucan test provides a clear overall picture of total mold exposure in your indoor environment.

- **Laboratory Testing Methods:** AirAnswers® in-house laboratory service uses validated molecular biological techniques, similar to what is used in a hospital reference lab, to detect (1→3)-β-d-glucan in your sample. Spore traps require identifying and counting spores on a slide with a microscope. Unlike AirAnswers® objective testing methods, spore analysis results may be different based on the analyst.

- **Sensitivity:** Our mold test measures small fractions of a carbohydrate, known as Beta-glucan or (1→3)-β-Dglucan, that are released into the air by germinating and growing mold. With our device, we are not only capturing spores, but we are also capturing hyphae and free-floating beta-glucan from growing molds. Our test is sensitive enough to detect mold that is growing behind walls and in places where mold is not detectable through sight or smell.

- **Can the device be used in high dust areas, like factories?**
  Yes, being that our air sampling technology captures airborne particles on steel electrodes vs. filters, there is no chance of clogging the AirAnswers® device or capture cartridge.

- **Does opening doors and windows impact SARS-CoV-2 testing?**
  We recommend people keep their exterior windows and doors closed during testing if they are typically closed during normal operation, as external airflow dilutes indoor levels of SARS-CoV-2.

Cartridge and Electrodes:

- **Can the cartridge be overloaded?**
  Our cartridge cannot be overloaded. There is no limited capacity as there is no filter to worry about clogging.

- **How long can you keep the cartridge out of the clamshell before it's contaminated?**
  We recommend that you keep the cartridge in the clamshell until you're ready to use it.

- **Can I leave the cartridge in the device before plugging in?** Yes

- **How do you ensure your cartridges are not contaminated during shipment?**
  Each cartridge is shipped in a clean plastic clamshell container inside of a sealed plastic bag.

- **What happens if I accidentally drop the cartridge when taking it out of the device?**
  If it is dropped on a clean, dry surface, and the electrodes do not come loose from the cartridge, then the cartridge should be usable. If dropped on a potentially dirty surface or if the electrodes come loose, you will need to repeat the run using another cartridge. Note that whichever way the cartridge falls, the stainless steel electrodes do not come in direct contact with the surface.

- **Why are the cartridges not reusable?**
  The electrodes are designed for single use because they are disassembled as part of the laboratory analysis process. Also, electrodes are not reusable as the surface is treated by an electropolishing process. Once used, the surface is compromised.

- **What quality procedures do you have in place?**
  To avoid contamination, once received in the lab, a cartridge is only exposed to the air within a biosafety cabinet class II and handled with gloves. A biosafety cabinet class II protects the cartridge from outside contamination and protects the lab analyst from exposure to the particulate matter collected on the cartridge. Clean conditions (e.g., PPE, air filtration, etc.) are used for cartridge manufacture, and rigorous manufacturing quality control testing is employed to ensure cartridge performance and guard against any cartridge contamination.

Laboratory:

I. General Questions:

- **Why do we have to send the cartridges to AirAnswers® laboratories?**
  Currently only AirAnswers® laboratory has the validated and quality-controlled equipment, procedures, and assays designed to analyze the bioaerosols captured by our cartridges.

- **How do I register my cartridge?**
  You can register your cartridge through your account. Through your account, you will choose your test, pay for your testing with a credit card, and view your results. If you don't have an account, contact your sales consultant, who will help you set one up.

- **What happens if I send my cartridge into the lab without registering it?**
  The laboratory will only start testing cartridges that have been registered. Turnaround time for results will not start until your cartridge is registered. If you send in your cartridge without registering, contact your sales consultant. Your sales consultant will help you with the registration process and once the cartridge is registered, your testing begins.

- **How long will the lab keep my sample?**
  60 Days

- **What if I want to add additional testing 61 days after the lab receives my sample?**
  We will dispose of all samples that are over 60 days-old. You will need to run another cartridge.

- **What is the difference between PCR and microscopy and why is qPCR better?**
  PCR is far more sensitive and specific for the detection of pathogens such as molds, viruses, and bacteria. It is an objective approach to mold analysis. Microscopy for spore identification is subjective and analyst dependent. **Limitations of microscopy used in analysis of spores captured by spore trap are as follows:**

  - Analysts can only identify intact spores – if spores are broken into pieces, they are not identifiable
  - Analysts cannot distinguish between Aspergillus and Penicillium spores – both types of spores look extremely similar. In mold spore reports, they are reported together as Asp/Pen.
  - Difficulty of analysts in distinguishing individual spores in an often densely crowded field

- Variability of analyst skill
- The inability of spore trap to capture the smaller airborne mold particles indicative of actively growing mold

What testing does AirAnswers® offer?
Beta-Glucan, Mold Genera Panel, Mycotoxin Panel, Opportunistic Fungal Pathogen Panel, Powdery Mildew, Allergens, and SARS-CoV-2

What is the turnaround time for lab testing?
Turnaround time depends on the testing selected:

- **24-Hours:** Beta-Glucan & Mold Genera
- **2-Days:** SARS-CoV-2, Powdery Mildew, Opportunistic Fungal Pathogen Panel
- **5-Days:** Mycotoxins, Allergens, Allergens & Beta-Glucan Panel, Comprehensive Allergen & Mold Assessment, and Total Mold Panel

If I test for (1→3)-β-d-glucan, can I add on other mold tests with the same cartridge?
Yes, your collected sample will be stored in our laboratory for future testing. We can easily test your stored sample for any additional mold-based menu items, within the 60-day storage period.

What does it mean when it says that the AirAnswers® detects more than 23 molds with 1 test?
The majority of molds, and those most commonly associated with household mold damage, produce beta-glucan. Our beta-glucan test can detect hundreds of molds with one test. With one result, you will receive the total exposure to fungal mass in your indoor environment.

I found black mold in my home and your test came-back positive for Beta Glucan, but negative for Stachybotrys. How could this happen?
Just because a mold is black, doesn't always mean that it is Stachybotrys charutum, the notorious toxic black mold. One of the most commonly found molds, Cladosporium, is also black. Other black molds include Alternaria and Ulocladium.

Does your beta-glucan test detect all molds?
No, Our beta-glucan test does not detect certain fungal species, such as Cryptococcus sp. and Blastomyces dermatitidis, which produce very low levels of (1→3)-β-D-Glucan or Zygomycetes, such as Absidia, Mucor and Rizopus, which are not known to produce (1→3)-β-D-Glucan.

- **Does your COVID test include all variants?**
  Samples are analyzed by RT-PCR using primers/probe sets and protocols designed and provided by the CDC (Center for Disease Control and Prevention). Testing has been adapted for the detection of airborne SARS-CoV-2. Our COVID testing does identify all variants of COVID. AirAnswers® has been diligent in confirming that new variants are detectable using our testing method, and constantly is on the alert for the emergence of new variants.

II. AirAnswers® Testing Menu Descriptions:

- **(1→3)-β-d-glucan:** AirAnswers® performs a sensitive test for the presence of (1→3)-β-d-glucan suspended in air. A vast majority of mold species have (1→3)-β-d-glucan as a part of their cell walls, spores, and as smaller particulate matter emitted into the air by germinating and growing molds. These smaller particles are more likely to remain airborne longer and penetrate deep into the lungs. AirAnswers® captures these smaller airborne particles and the (1→3)-β-d-glucan they contain is selectively measured by AirAnswers® proprietary assay. With one result, you will receive the total exposure to fungal mass in your environment. Levels of (1→3)-β-d-glucan in the air are known to correlate with fungal mass and severity of symptoms. In homes with active mold growth, (1→3)-β-d-glucan was found in sizes ranging from 18 to 0.18 micron. This means that a large fraction was in particles and fragments smaller than spores and might be missed by other methods

- **Powdery Mildew:** Powdery mildew is a fungal disease affecting plants, characterized by white or grayish patches on infected plants. Powdery mildew is one of the most widespread of plant fungal diseases affecting cannabis and other agricultural industries. To detect powdery mildew, we use a quantitative polymerase chain reaction (qPCR). Our state-of-the- art testing identifies 4 of the most common species of powdery mildew affecting cannabis crops: Erysiphe Necator, Govinomyces ambrosia, Golvinomyces spedicius, and Podesphaera xanthii.

- **Mold Genera and Mycotoxins:** To detect airborne molds and mycotoxin we use the gold standard of detection, a quantitative polymerase chain reaction (qPCR). This is the most sensitive means available for the detection of active mold and the generation of mycotoxin. This lets us not only identify that mold and mycotoxins are present, but also give an indication of the mold and mycotoxin levels in the air.

Allergens:

- **Dust Mites:** Dust mite allergens are a common trigger of allergy and asthma symptoms. While they can be found throughout the house, these microscopic creatures thrive in warm, humid environments such as bedding, upholstered furniture and carpeting. Because so much time is spent in the bedroom, it is essential to reduce mite levels there.

- **Cats (Fel d 1) & Dogs (Can f 1):** Contrary to popular opinion, there are no “hypoallergenic” breeds of dogs or cats. That is because people are not allergic to an animal’s hair, but to an allergen found in the saliva, dander (dead skin flakes) or urine of an animal with fur. Keeping an animal outdoors is only a partial solution since homes with pets in the yard still have higher concentrations of animal allergens. Ask your allergist to determine if you are allergic to animals.

- **Cockroaches (Bla g 2):** Cockroaches are often found in the homes of densely populated urban areas, schools or commercial buildings, but these creatures can lurk almost anywhere. This does not mean that you have a dirty house or living area.

- **Mouse (Mus m 1):** Mouse allergens are found in saliva, dander (dead skin flakes) or urine. If mouse allergen is found in your home, this doesn’t necessarily mean that you have an infestation of rodents in your home. Mouse and rat allergen could be making its way into your home through an open window or transferred by a pet after coming in from outside.

- **Pollen (Weed (Amb a 1), Grass (Phl p 5), & Tree (Bet v 1):** Pollen are tiny grains needed to fertilize many kinds of plants. Pollen from plants with colorful flowers, like roses, usually do not cause allergies. These plants rely on insects to transport the pollen for fertilization. On the other hand, many plants have flowers which produce powdery pollen that are easily spread by wind. These culprits cause allergy symptoms.

Mold Genera Panel:

- **Aspergillus:**
  Commonly found in soil, decaying vegetation, and in house dust. Aspergillus fumigatus is the most common cause of aspergillosis. Exposure can lead to severe infection in immunocompromised individuals. By measuring the genes common to a genus of mold, we are less likely to miss the presence of a particular mold species within that genus. The Aspergillus test detects the following species of Aspergillus:

  Aspergillus fumigatus, Aspergillus niger, Aspergillus brunneoviolaceus, Aspergillus aculeatus, Aspergillus nomiae, Aspergillus sydowii, Aspergillus flavus, Aspergillus versicolor, Aspergillus tempicola, Aspergillus japonicus, Aspergillus nidulans, Aspergillus terrus, Aspergillus fischeri, Aspergillus neoellipticus, Aspergillus aureoles, Aspergillus udagawae, Aspergillus igneus, Aspergillus viridinutans, Aspergillus wyomingensis, Aspergillus oerlinghausenensis, Aspergillus tsurutae, Aspergillus brevistipitatus, Aspergillus waksmanii, Aspergillus pseudoviridinutans, Aspergillus shendaweii, Aspergillus siamensis, Aspergillus botucatensis, Aspergillus primulinus, Aspergillus galapagensis, Aspergillus aureoluteus, Aspergillus duricaulis, Aspergillus fumigatiaffinis, Aspergillus lentulus, Aspergillus pernambucoensis, Aspergillus fumisynnematus, Aspergillus neoglaber, Aspergillus laciniosus, Aspergillus assulatus, Aspergillus ferenczii, Aspergillus brevipes, Aspergillus coreanus, Aspergillus hiratsukae, Aspergillus nishimurae, Aspergillus turcosus, Aspergillus multiplicatus, Aspergillus papuensis, Aspergillus spathulatus, Aspergillus spinosus

- **Penicillium:**
  Associated with decaying food products, Penicillium includes over 300 species of mold. Penicillium can be found on cellulose materials inside homes and buildings and can even grow in environments of relative low humidity. The Penicillium test detects the following species of Penicillium:

  Penicillium citrinum, Penicillium onobense, Penicillium malochii, Penicillium chrysogenum, Penicillium vanluykii, Penicillium rubens, Penicillium oxalicum, Penicillium philippinense, Penicillium mexicanum, Penicillium lilacinoechinulatum, Penicillium ibericum, Penicillium turbatum, Penicillium thomii, Penicillium glabrum, Penicillium magnielliptisproum, Penicillium westlingii, Penicillium angulare, Penicillium malodoratum, Penicillium subspinulosum, Penicillium montanense, Penicillium lividum, Penicillium fuscum, Penicillium cosmopolitanum, Penicillium adametzii, Penicillium herquei

Zero Tolerance Molds: The following 5 molds are considered “Zero Tolerance” molds. They can be highly toxic to some individuals. If found, they require immediate remediation.

- **Stachybotrys:**
  Stachybotrys grows on products with high cellulose content, such as fiberboard, wood, and paper. The most notorious species, Stachybotrys chartarum, aka “black mold”, is associated with moisture from water damage, leaks, or flooding.

- **Fusarium:**
  Found in the air, soil, and on plants, Fusarium includes 20 species of mold. Some species of Fusarium can cause mycotoxicosis and severe disease, if digested. Like Stachybotrys, Fusarium can be associated with severe water damage from leaks, broken pipes, and flooding. The Fusarium test detects the following species of Fusarium:

  Fusarium oxysporum, Fusarium tardichlamydosporum, Fusarium graminarium, Fusarium culmorun, Fusarium proliferatum, Fusarium colani, Fusarium nygamai, Fusarium commune, Fusarium vanettenii, Fusarium sacchari, Fusarium incarnatum, Fusarium flagelliforme, Fusarium verticillioides, Fusarium tonkinense, Fusarium tricinctum, Fusarium sporotrichioides, Fusarium sambucinum, Fusarium venaceum, Fusarium iranicum, Fusarium torulosum, Fusarium acuminatum, Fusarium nurragi, Fusarium fujikuroi, Fusarium witzenhausenense, Fusarium gamsii, Fusarium reticulatum

- **Trichoderma:**
  Trichoderma is the most common naturally occurring mold in the ecosystem and is found to be useful in fields of industry and agriculture. It can be found in carpets, furniture, wallpaper, and on wood, if moisture conditions are high enough. Some species of Trichoderma are more resistant to anti-fungal drugs than many molds which poses a threat to immunocompromised individuals. The Trichoderma test detects the following species of Trichoderma:

  Trichoderma asperellum, Trichoderma harzianum, Trichoderma afroharzianum, Trichoderma virens, Trichoderma atroviride, Trichoderma brevicompactum, Trichoderma lixii, Trichoderma rugulosum, Trichoderma

koningiopsos, Trichoderma crissum, Trichoderma anaharzanum, Trichoderma ghanense, Trichoderma xixacum, Trichoderma carribaeum, Trichoderma dorothopsos, Trichoderma texanum, Trichoderma aggressivum

- **Memnoniella:**
  Memnoniella, a close relative of the infamous Stachybotrys molds, is a mold that commonly develops on water damaged construction material. Like Stachybotrys, Memnoniella grows on materials with cellulose content, in the presence of high moisture. The test for Memnoniella detects Memnoniella echinate.

- **Chaetomium:**
  Most commonly found in decaying plants, animal dung, and in damp soil, Chaetomium can grow on water damaged materials with high cellulose content. Many species can cause severe respiratory disease in immunocompromised individuals. The Chaetomium test detects the following species of Chaetomium:

  Chaetomium globosum, Chaetomium anastomosans, Chaetomium cruentum, Chaetomium camelliae, Chaetomium citrinum

Mycotoxin Panel:

Mycotoxins are naturally occurring biomolecular toxins produced by certain molds (fungi). According to NORMI (National Organization of Remediators and Mold Inspectors), some mycotoxins can cause cancer, some suppress the immune system, and others can attack the liver and kidneys.

- **Trichothecene:**
  Trichothecenes are produced by Trichoderma and Stachybotrys, such as Stachybotrys chartarum (black mold). We detect the following Trichothecenes: Deoxynivalenol, Diacetoxyscirpenol, HT-2 mycotoxins, Nivalenol,T-2 mycotoxins.

- **Ochratoxin:**
  Ochratoxins are produced by some Aspergillus and Penicillium species of molds, such as Aspergillus niger and Penicillium verrucosum. We detect Ochratoxin A.

- **Aflatoxin:**
  Aflatoxins are highly liver carcinogenic. They are mainly produced by some species of Aspergillus, such as Aspergillus flavus and Aspergillus parasiticus. We detect Aflatoxin B1 and B2.

- **Fumonisin:**
  Fumonisins are environmental toxins produced by Fusarium species, such as Fusarium moniliforme and Fusarium proliferatum. We detect fumonisin B1.

Opportunistic Fungal Pathogen Panel:

Immunocompromised individuals have weakened immune systems. Due to age or to specific medical conditions, this population is left vulnerable to infection by the following panel of fungi:

- **Aspergillus fumigatus & Aspergillus niger:**
  Aspergillus fumigatus and Aspergillus niger are both common species of fungi in the genus, Aspergillus. Both types of fungi can cause disease in individuals with immunodeficiencies. Diseases associated with Aspergillus fumigatus include chronic pulmonary aspergillosis, invasive aspergillosis, a life-threatening disease which can infect multiple organs, pneumonitis, aspergilloma, allergic asthma, and hypersensitivity. In rare circumstances, Aspergillus niger can also cause aspergillosis. Otomycosis, a fungal ear infection, which causes severe pain, hearing loss, and damage to the ear canal, can also be attributed to Aspergillus niger infections.

- **Mucor:**
  Mucor is a genus of fungi which comprises approximately 40 species. Breathing in some species of Mucor can cause severe disease in immunocompromised individuals, known as mucormycosis. Mucormycosis is a severe and potentially fatal fungal infection starting in the sinuses or lungs. It is especially serious due to the fast-spreading nature of the infection. Without treatment, it can rapidly spread throughout the body. Mucor does not contain beta-glucan, (1→3)-β-d-glucan, so will not be detected by our beta - glucan assay.

- **Candida albicans:**
  Candida albicans is the most common species of yeast that is associated with infection in humans. In immunocompromised individuals, Candida can cause invasive candidiasis, a serious infection that can enter the bloodstream and infect internal organs. According to the Centers for Disease Control and Prevention, invasive candidiasis is one of the most common causes of bloodstream infections in hospitalized patients and can lead to long hospital stays and death. Being that Candida is a yeast, it will not be detected by our beta-glucan test, which is specific for mold.

ACAC Collaboration:

ACAC (American Council for Accredited Certification) - Environmental Allergen Certification (CEAC/CEAI/CEAT): In 2019, AirAnswers® was part of the steering committee responsible for the creation of this IAQ certification

https://www.acac.org/allergencertification

Supporting Science & Patents:

All peer-reviewed publications and environmental chamber studies are available upon request

- **Patents:**
  US 9360402, 8038944, 9216421, 9481904, 9618431, 10835891 as well as other patents issued and pending in the United-States and in foreign jurisdictions.

- **Collaborating Institutions:**
  Our research is published in major peer-reviewed scientific journals and has been scientifically validated in hundreds of homes, commercial institutions, environmental chamber studies, and with leading allergists, researchers, and institutions, including the University of Chicago, Johns Hopkins University, Harvard, and Argonne National Laboratories.

List of AirAnswers' Publications & Publication Using AirAnswers® Technology:

1. Richardson, M., et al., Concurrent measurement of microbiome and allergens in the air of bedrooms of allergy disease patients in the Chicago area. Microbiome, 2019. 7(1): p. 82

Abstract:
The particulate and biological components of indoor air have a substantial impact on human health, especially immune respiratory conditions such as asthma. To better explore the relationship between allergens, the microbial community, and the indoor living environment, we sampled the bedrooms of 65 homes in the Chicago area using 23 the patient-friendlyInspirotec electrokinetic air sampling device, which collects airborne particles for characterization of both allergens and microbial DNA. The sampling device captured sufficient microbial material to enable 16S rRNA amplicon sequencing data to be generated for every sample in the study. Neither the presence of HEPA filters nor the height at which the air sampling device was placed had any influence on the microbial community profile. A core microbiota of 31 OTUs was present in more than three quarters of the samples, comprising around 45% of the relative sequence counts in each bedroom. The most abundant single organisms were Staphylococcus, with other core taxa both human and outdoor-associated. Bacterial alpha diversity was significantly increased in bedrooms that reported having open windows, those with flowering plants in the vicinity, and those in homes occupied by dogs. Porphyromonas, Moraxella, Sutterella, and Clostridium, along with family Neisseraceae, were significantly enriched in homes with dogs; interestingly, cats did not show a significant impact on microbial diversity or relative abundance. While dog allergen load was significantly correlated with bacterial alpha diversity, the taxa that significantly correlated with allergen burden did not exclusively overlap with thoseenriched in homes with dogs. Alternaria allergen load was

positively correlated with bacterial alpha diversity, while Aspergillus allergen load was negatively correlated. The Alternaria allergen load was also significantly correlated with open windows. Microbial communities were significantly differentiated between rural, suburban, and urban homes and houses that were physically closer to each other maintained significantly more similar microbiota. We have demonstrated that it is possible to determine significant associations between allergen burden and the microbiota in air from the same sample and that these associations relate to the characteristics of the home and neighborhoods.

2. Gordon, J., et al., Validation of a novel sampling technology for airborne allergens in low-income urban homes. Annals of Allergy Asthma & Immunology, 2018. 120(1): p. 96-+.

Abstract:

Background: For many years, vacuumed dust collection has been the preferred method for assessing allergenexposure because of its ease of collection. Ideally, allergen exposure assessment should be based on measurement of airborne concentrations as this is a truer reflection of airway exposure, but dust collection has been used as a surrogate. Available air sampling methods have been reported as cumbersome, expensive and time consuming. Therefore dust has remained as the standard method for measuring allergen exposure. Inspirotec Inc. has developed a simple plug-in device for airborne allergen capture. It is compact, unobtrusive, and requires no technical expertise to operate. Though various methods of air sampling have been compared with dust collection, the Inspirotec sampler, has yet to be compared with other air dust and air sampling methods for allergen capture. The purpose of this study was to validate the performance of the Inspirotec air sampler against other available reference methods. In this study, the Inspirotec sampler has been placed side-by-side in homes with PM10 air filters and dust collection by vacuuming. This work is part of an ongoing trial where multiple analyses are performed in urban homes at baseline, 3 months, and 6 months.

Results & Conclusions: The Inspirotec sampler correlates with both PM10 and dust collectors for cat (Feld 1), mouse (Mus m 1) and dog (Can f 1) allergens. All 3 were most frequently found in homes. More positives and lower detection levels were found for all allergens, with the exception of birch pollen, by the Inspirotec sampler versus the PM10 due to large air volumes sampled per unit time by Inspirotec samplers. Less positives were found for cat, dog, dust mite, roach and mouse by the Inspirotec sampler versus dust collection due to more concentrated allergens in the dust. Birch pollen was found outside of the pollen season by both Inspirotec and PM10 for both outdoor pollen counts and indoor pollen allergen concentrations. The Inspirotec sampler showed Phl p 5 allergens within pollen season, while majority of positives were found outside of the season for the PM10 sampler.

3. Gordon, J., et al., Bedroom exposure to airborne allergens in the Chicago area using a patient-operated sampling device. Annals of Allergy Asthma & Immunology, 2018. 121(2): p. 211-+.

Abstract:

Background: In current practice, allergens in vacuum-collected dust are used as surrogates for inhalable allergens. We developed an air-sampling device that can be used by patients for direct measurement of airborne allergen concentrations in their own homes.

Objective: To demonstrate the use of this device to establish allergen concentration reference ranges in a target population and to evaluate associations between patient-reported information and measured allergen concentrations.

Methods: Patients from 5 allergist's practices in the Chicagoland region were provided with instructions, questionnaires, informed consent forms, and samplers to run for 5 days in their bedrooms. Samples were collected from cartridges and assayed by multiplex immunoassays for 12 common household allergens and enzyme-linked immunosorbent assay for ragweed.

Results: Unique allergen profiles were obtained for 102 patient homes. Samples with allergen concentrations above the limit of detection were as follows: total dust mite, 28%; cat, 61%; dog, 64%; mouse, 12%; rat, 0%; cockroach, 4%; Alternaria, 6%; Aspergillus, 21%; birch pollen 1%; grass, 8%; and ragweed, 5%. Of those, 75 completed questionnaires, providing meta-data for further analysis. Pet allergens correlated significantly with the number of pets owned. Humidity correlated with dust mite allergens, open windows with Alternaria and mouse allergens, and high-efficiency particulate air filter use with reduced levels of several allergens. Many other variables showed no significant correlations.

Conclusion: The combination of ease of use, high air-sampling rate, and sensitive immunoassays permitted the measurement of airborne allergen concentrations in homes and establishment of reference ranges. Patient-reported information permitted identification of factors that could relate to allergen concentrations and suggested remedial measures.

4. Gordon, J., P. Gandhi, and P. Detjen, High Sensitivity Measurements of Airborne Allergens Using a Patient-Operated Sampling Device: A New Technology Reveals Indoor Aerobiome. Journal of Allergy

Abstract:

Background: In current practice, allergens in vacuum-collected dust are used as surrogates for inhalable allergens. We developed an air-sampling device that can be used by patients for direct measurement of airborne allergen concentrations in their own homes.

Objective: To demonstrate the use of this device to establish allergen concentration reference ranges in a target population and to evaluate associations between patient-reported information and measured allergen concentrations.

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Conclusion: The combination of ease of use, high air-sampling rate, and sensitive immunoassays permitted the measurement of airborne allergen concentrations in homes and establishment of reference ranges. Patient-reported information permitted identification of factors that could relate to allergen concentrations and suggested remedial measures.

5. Gordon J, Detjen P, Kelso D, Gandhi P. A new patient-operated sampling device for measurement of aeroallergens. Ann Allergy Asthma Immunol. 2016;116:475–476. and Clinical Immunology, 2017. 139(2): p. AB120-AB120.

Abstract:

Background: A simple plug-in device for air sampling, which is inconspicuous and requires no technical skill for operation in a variety of settings, has been developed. Performance compared with a reference method for mold species and bacterial microbiome analysis was examined. A study using this system for common allergen profiling in the homes of 22 patients has been previously presented. Selection criteria were homes of patients with allergic rhinitis and/or allergic asthma and ownership of cat, dog, or both.

Methods: Test results from samples in which patients ran the devices in the bedroom for 24 hours and 7 days. Patients executed Health Insurance Portability and Accountability Act and informed consent agreements. They were given a questionnaire with particulars about patient home environment and avoidance measures, including pet avoidance measures.

Results: All patients successfully completed runs without supervision. The device incorporates a removable cartridge with releasable electrodes. The released stainless steel electrode strips

are extracted directly with a standard buffer. The extracts were analyzed with the MARIATM common household allergen multiplex assay6,7 by Indoor Biotechnologies (Charlottesville,VA). All patients reporting cat ownership had some measurable level of cat allergen in the bedroom, except patient 20, who was apparently successful in excluding the cat from the bedroom and eliminated measurable airborne allergen. Homes with no dog had no detectable dog allergen, with one exception.

Conclusions: This data can be used to test for consistency with patient-reported presence or absence of pets and efficacy of reported mitigation measures. The ability of patients to generate allergen profiles of the breathable air in their own homes provides an otherwise unavailable adjunct to patient care.

6. Gordon, J., et al., A simple novel device for air sampling by electrokinetic capture. Microbiome, 2015. 3.

Abstract:

Background: A variety of different sampling devices are currently available to acquire air samples for the study of the microbiome of the air. All have a degree of technical complexity that limits deployment. Here, we evaluate the use of a novel device, which has no technical complexity and is easily deployable.

Results: An air-cleaning device powered by electrokinetic propulsion has been adapted to provide a universal method for collecting samples of the aerobiome. Plasma-induced charge in aerosol particles causes propulsion to and capture on a counter-electrode. The flow of ions creates net bulk airflow, with no moving parts. A device and electrode assembly have been re-designed from air-cleaning technology to provide an average air flow of 120 lpm. This compares favorably with current air sampling devices based on physical air pumping. Capture efficiency was determined by comparison with a 0.4 μm polycarbonate reference filter, using fluorescent latex particles in a controlled environment chamber. Performance was compared with the same reference filter method in field studies in three different environments. For 23 common fungal species by quantitative polymerase chain reaction (qPCR), there was 100 % sensitivity and apparent specificity of 87 %, with the reference filter taken as “gold standard.” Further, bacterial analysis of 16S RNA by amplicon sequencing showed equivalent community structure captured by the electrokinetic device and the reference filter. Unlike other current air sampling methods, capture of particles is determined by charge and so is not controlled by particle mass. We analyzed particle sizes captured from air, without regard to specific analyte by atomic force microscopy: particles at least as low as 100 nM could be captured from ambient air.

Conclusions: This work introduces a very simple plug-and-play device that can sample air at a high-volume flow rate with no moving parts and collect particles down to the sub-micron range. The performance of the device is substantially equivalent to capture by pumping through a filter for microbiome analysis by quantitative PCR and amplicon sequencing.

7. John Crowe, PhD 1 ; Andy T. Schnaubelt, PhD 2 ; Scott SchmidtBonne, MA 1 ; et al, Assessment of a Program for SARS-CoV-2 Screening and Environmental Monitoring in an Urban Public School District,

- AirAnswers® was used in this study in collaboration with the University of Nebraska and Josh Santarpia

- Findings Pertaining to AirAnswers® - SARS-CoV-2 RNA was detected in the air with AirAnswers® in two choir rooms and from surface testing in one choir room

Abstract:

Scalable programs for school-based SARS-CoV-2 testing and surveillance are needed to guide in-person learning practices and inform risk assessments in kindergarten through 12th grade settings.

Objectives To characterize SARS-CoV-2 infections in staff and students in an urban public school setting and evaluate test- based strategies to support ongoing risk assessment and mitigation for kindergarten through 12th grade in-person learning.

Design, Setting, and Participants This pilot quality improvement program engaged 3 schools in Omaha, Nebraska, for weekly saliva polymerase chain reaction testing of staff and students participating in in-person learning over a 5-week period from November 9 to December 11, 2020. Wastewater, air, and surface samples were collected weekly and tested for SARS -CoV-2 RNA to evaluate surrogacy for case detection and interrogate transmission risk of in-building activities.

Main Outcomes and Measures SARS-CoV-2 detection in saliva and environmental samples and risk factors for SARS-CoV-2 infection.

Results A total of 2885 supervised, self-collected saliva samples were tested from 458 asymptomatic staff members (mean [SD] age, 42.9 [12.4] years; 303 women [66.2%]; 25 Black or African American [5.5%], 83 Hispanic [18.1%], 312 White [68.1%], and 35 other or not provided [7.6%]) and 315 students (mean age, 14.2 [0.7] years; 151 female students [48%]; 20 Black or African American [6.3%], 201 Hispanic [63.8%], 75 White [23.8%], and 19 other race or not provided [6.0%]). A total of 46 cases of SARS-CoV-2 (22 students and 24 staff members) were detected, representing an increase in cumulative case detection rates from 1.2% (12 of 1000) to 7.0% (70 of 1000) among students and from 2.1% (21 of 1000) to 5.3% (53 of 1000) among staff compared with conventional reporting mechanisms during the pilot period. SARS-CoV-2 RNA was detected in wastewater samples from all pilot schools as well as in air samples collected from 2 choir rooms. Sequencing of 21 viral genomes in saliva specimens demonstrated minimal clustering associated with 1 school. Geographical analysis of SARS-CoV-2 cases reported district-wide demonstrated higher community risk in zip codes proximal to the pilot schools.

Conclusions and Relevance In this study of staff and students in 3 urban public schools in Omaha, Nebraska, weekly screening of asymptomatic staff and students by saliva polymerase chain reaction testing was associated with increased SARS -CoV-2 case detection, exceeding infection rates reported at the county level. Experiences differed among schools, and virus sequencing and geographical analyses suggested a dynamic interplay of school-based and community-derived transmission risk. Collectively, these findings provide insight into the performance and community value of test-based SARS-CoV-2 screening and surveillance strategies in the kindergarten through 12th grade educational setting.

Environmental Chamber Studies:

1. Aerosol Research and Engineering Laboratories: Characterization of Particle the Collection Efficiency for the AirAnswers® Air Sampling Device Using Fluorescent PSL Microspheres

- **Background:** AirAnswers® by Inspirotec, Inc., is the only commercial air sampling device that has the capability to collect ultrafine particles (0.1um) as well as viruses, including airborne COVID, bacteria, fungi, and allergens. AirAnswers® is a plug-n-play device with no moving parts that utilizes ion particles to generate air flow through the device. Plasma-induced charge in aerosol particles causes propulsion to and capture on acounter-electrode. The flow of ions creates net bulk airflow, with no moving parts. This study's purpose was to quantify the collection efficiency for the AirAnswers. device at three (3) different size ranges, two (2) different temperature ranges, two (2) different relative humidity levels at two (2) different challenge concentrations with each unique condition tested with three (3) replicates. The collection efficiency testing used fluorescent monodispersed polystyrenelatex microspheres (PSL's) as the test article. PSL microspheres, due to their density and dielectric potentials are excellent surrogates for biological aerosols.

- **Methods:** A 1m3 dynamic aerosol test chamber was used to conduct the study. The chamber was operated dynamically with continuous aerosol introduction and evacuation to maintain a steady-state concentration during the sampling portion of the trial. Once steady- state concentration was achieved, sampling for both the AirAnswers. and reference filter were started. After a period of time the samplers were stopped, the chamber evacuated, and the AirAnswers® collection electrodes and filter samples were recovered. The samples were extracted in DI water and 0.05% tween 80 solution (non-ionic surfactant) and the resultant solution was quantified using a fluorometer to determine microsphere concentrations. Calibrated pipettes were used to serially dilute samples prior to fluorimetry measurements. Direct comparison between the reference filter and the AirAnswers® devices were used to show collection efficiency for each trial and group average +/- standard deviations was computed for all individual PSL sizes for each unique environmental condition.

Results: The results of the testing show that the AirAnswers® device yielded the highest collection efficiency when tested with the 0.1um particle size. The testing also showed higher collection efficiency at the higher relative humidity levels. The temperature didn't have a large effect on the collection performance of the device.

2. MRI Global: Evaluation of a Bio-Aerosol Sampler in Collection Efficiency of Aerosolized SARS-CoV-2

- **Objective:** The objective of this project was to measure the efficacy of Inspirotec Inc. AirAnswers® Bio-aerosol collector in airborne collection of aerosolized SARS-CoV-2

- **Methods:** Aerosol testing was performed using an aerosol test system fabricated out of Plexiglas. The test system was housed in the Class III Biosafety Cabinet for all conducted tests. The aerosol containment system has internal dimensions of 2.5ft high × 3.5ft wide × 1.5ft deep, with a displacement volume of approximately 370 liters or 13.1 cubic feet. The bio-aerosol test system is fabricated for nebulizer adaptation, aerosol and sample dilution, air displacement filtration, air supply regulation and control, exhaust flow regulation, aerosol sampling, particle size measurement, and temperature and humidity monitoring. Aerosol generation and sampling system pressures and flow rates were monitored and controlled for maintaining reproducible test conditions using calibrated digital mass flow meters and controllers. SARS-CoV-2 aerosol nebulizer generation was provided with flow and pressure regulated tank supplied breathing grade air.

- **Conclusion: Based on these experiments, we conclude that the AirAnswers® devices are very consistent and reproducible in aerosol collection and have a high rate of aerosol particle collection. The results showed reproducible collection efficiencies of approximately 16 percent. The results also show that the AirAnswers® electrode analysis reflects a very low percentage of viable collection in relation to the viable viral fraction analyzed**