WHITE PAPER

Salvus Detection Technology Rapidly Delivers Laboratory-Quality Results for Detection of Chemicals and Biologicals in a Handheld Unit

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Salvus Detection Technology Can Ease the Burden of Detecting Your Concerns

In a world that constantly seeks advancement and improvement – faster, better products, at higher quality and lower cost – diagnostic tools have paramount importance to institutions seeking to stay ahead of the curve. Whether working with spreadsheets to track inspected inventories in a business or measuring water components to keep customers supplied with clean drinking water, the ability to quickly receive and analyze results and execute decisions based upon those results is already differentiating which operatives will be industry leaders and those that will stagnate. This is especially true of those companies working in the chemical and biological sectors, where quick detection of impurities and pathogens can make a difference of millions of dollars in protection of assets and prevention of outbreaks or contamination [1].

Figure 1: Commercially ready handheld Salvus technology device
Sizing and basic layout of the handheld device capable of swapping out cartridges to detect chemical and biological targets in fluid media. Users simply insert the cartridge, introduce sample, and use the touchscreen to run the necessary test parameters to receive qualitative or quantitative analyte results in minutes.

Despite this need for actionable detection results, in many cases, current detection methods have several limiting characteristics:

- Require specialized equipment and highly trained personnel
- Take days or weeks to provide results
- Lack necessary measurement capabilities (specificity, sensitivity, accuracy)
- Are exceedingly expensive

For professionals in various sectors in need of an easy-to-use, sensitive, specific point-of-use method to detect analytes that impact their field of expertise, including water quality management, environmental sciences, agricultural, industrial, food processing and safety, and healthcare, we at Salvus have developed a detection technology that overcomes the limitations of

current available testing methods, providing for in-field decisions (see Figure 1). The Salvus technology provides:

- Easy-to-use touch screen interface and sample introduction even for new users
- Results on the scale of minutes
- Underlying technology that allows for high sensitivity and specificity
- Favorable cost to value relationship
- Other convenient capabilities mentioned later in this document

Consider for a moment the position of dairy farmers trying to monitor for bovine mastitis in their ruminants. Though the sub-clinical (not visually evident) form of this disease costs dairy farmers in the United States alone greater than $1 billion each year, current point-of-use tests for dairy farmers cover a troubling range of sensitivities and impart variable results, while the potentially more sensitive and accurate laboratory tests require dramatically higher cost and turn-around time [2,3]. And dairy farmers are not the only ones who lack accessibility to a test providing fast, sensitive results. Professionals across industries such as healthcare, agriculture, drug identification, and water management also suffer from unnecessary losses of time, product, and money from having to rely on their current detection options [4,5]. Now, imagine the dairy farmers can easily screen for sub-clinical bovine mastitis using a device at hand which reduces that $1 billion loss and improves animal health and food production. This is only one of many potential applications of the Salvus technology. Suitable for assessing a wide range of biological and chemical agents (see Table 1), including pathogens, chemical contaminants, and biological markers, this new detection technology demonstrates cross-industry applicability for a multitude of target analytes [6-8].

Table 1: Select Examples of Target Analytes and Their Detection Measurements Using Waveguide Interferometry*

Targets	Measurement
SARS-CoV2 Antigen	$<10x10^4$ copies/ml
SARS-CoV2 Antibody	Titer <439 at 1,000-fold dilution
Salmonella	$<5x10^5$ cells/ml
Y. pestis F1 Antigen (Plague)	<100 pg/ml (ppt)
Ricin A Chain	<830 pg/ml (ppt)
Chlorine in Poultry Process Water	<500 ppb
2,4-D	<100 ppb
Dicamba	<500 ppb
Mesotrione	<200 ppb

** Test results of collaborators at Georgia Tech Research Institute*

Salvus Detection Technology Incorporates Well-Established Sciences in an Innovative Platform

The Salvus technology uses a small sensor that combines features of optical, chemical, and biological sciences to achieve target detection with its advantageous characteristics of speed, sensitivity, and specificity. In terms of optics, the underlying technology combines

interferometers with waveguides, which will be further described shortly. Different chemical reactions can be used to apply a receptor layer that interacts between the waveguide and the measured sample to allow for measurement. The receptors can be chemical or biological in variety and can detect chemical or biological targets in fluid media including liquids and air.

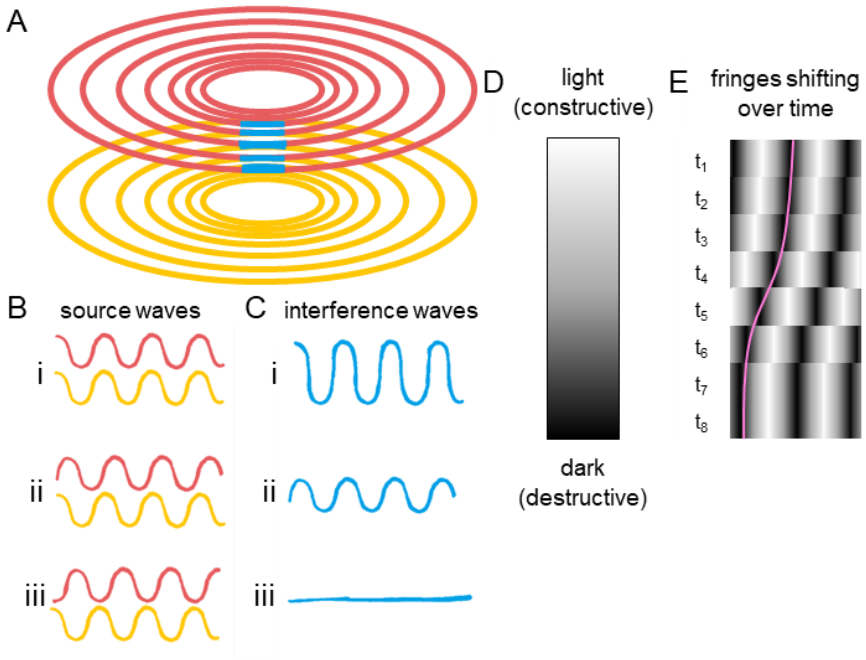

Figure 2: Representation of source waves combining to form interference waves and their respective resulting signals in Young's planar interferometry
A) Source waves are shown in red and yellow; where they meet and interfere with each other is shown in blue. **B)** Two-dimensional view of source waves with the shifting phase wave in red on top and the reference, stationary phase wave in yellow on the bottom**. (i)-(iii)** show waves in phase, out of phase, and perfectly out of phase, respectively. **C)** Interference waves in blue resulting from the combination of the source waves in B). As the red top wave shifts from being in phase with the yellow bottom wave, then to out of phase, and finally to perfectly out of phase, the resulting wave moves from constructive interference with a greater intensity than the source waves **(i)** to being near the same intensity as the source waves **(ii)** to destructive interference where the signals cancel each other out **(iii)**. **D)** The visual depiction of the light intensities resulting from the interference waves, ranging from light - due to constructive interference (waves combine to form greater intensity) - to dark - due to destructive interference (waves combine and cancel). The light and dark intensities create light and dark fringes. **E)** These fringes shift as the red wave's phase shifts while the yellow wave's phase remains stationary. If the red wave's phase stops shifting, the fringes will stop shifting as well. This is shown at discrete times $t_1 - t_8$, where a slight shift occurs from t_1 through t_3, then a slightly larger shift occurs from t_4 through t_6, and no shift occurs from t_7 to t_8. The pink line shows what the shape of a recorded response from such shifts might look like.

The interferometric method implemented is Young's planar interferometry. This can be conceptually explained by using waves in water as a, perhaps, more comprehensible analogy. For example, picture two people standing by a lake, and each throws a stone into the water. The stones form similar ripple patterns of waves in the water that travel away from their sources and eventually intersect, causing other waves to form as they interfere with each other (see Figure 2). Depending on how the peaks and troughs of the ripples interfere with each other, or what their phases are relative to each other, the resulting waves could be bigger (if they are near or of the same phase), smaller (if they are out of phase), or even cancel each other out completely (if they are perfectly out of phase). Now say there are some leaves that drift over in the water near one of the wave's paths, and the leaves slow down the wave's propagation relative to the other unhindered wave. This slowdown would result in a change in the timing of when the peaks and troughs of the slowed wave interfere with the other wave's peaks and troughs, causing a phase shift in the interference wave. This shift

could be measured relative to the waves' original interference wave before the leaves made their way into the picture, allowing the phase change due to the leaves to be calculated. The intersection when it comes to waves of light, rather than water, forms what is referred to as interference fringes (light and dark), which is what is optically measured to detect a target analyte. As the properties of the fluid near the surface of the sensor - along the path over which the light wave travels - change, those changes can affect how quickly or slowly the two waves intersect, measured as a shift in the interference wave's phase. That shift in phase is then used to measure the amount or presence of whatever led to that shift, such as chemical or biological matter in the fluid.

Waveguides are materials that guide and propagate light. In the Salvus technology, waveguides are used to form the path – the path over which the light wave travels – for the light used in interferometric measurements. If you have ever seen the reflection of an object in water that is just below the surface of the water, such a scenario is a good example for waveguiding. (If you have not seen this, search for "underwater reflection" in your web browser, and you are likely to find many sample images). At the interface of two materials with different material properties, specifically in terms of refractive index (the ratio of light velocity in a vacuum, where it is unhindered by any matter, to light velocity in a specified medium, where it is hindered by the matter in that medium), light will be reflected from that interface as the light travels through it. Some of the light will also propagate outside and just above the surface of the waveguiding material, forming what is called an evanescent wave. The light of the evanescent wave is the light that the Salvus technology uses to measure analytes as they bind to the sensor within the evanescent field above the waveguide.

The sensor of the detection device uses glass as a substrate with channels etched into it, each with a parallel "buried" channel to function as a reference that is not exposed to the fluid sample (see Figure 3). All the channels have waveguide material deposited on the glass substrate with another layer of glass sandwiching the waveguide material. The etched channels have a much thinner layer of top glass and are coated with a chemical or biological receptor to detect a specific analyte. During detection, laser light is directed to one end of the waveguide, where there is a precise pattern of gratings with dimensions necessary to direct the laser light source's specific wavelength to the waveguide material on the channels (in the visible light spectrum, red has a long wavelength while purple has a short wavelength, which is why we see them as different colors). After the light has traversed the waveguide material, generating the evanescent wave in the small volume above it where the analyte receptor is coated to the waveguide, the light exits through another set of gratings that directs it to a surface over which a camera is aimed to record the fringe patterns generated by the evanescent waves. The camera acts as a transducer that converts the patterns into interpretable electrical signals [9]. This conversion allows for mathematical functions to be performed on the data to quickly transform and analyze the data. Fourier transforms are used with cyclical (waves) or circular data to, in essence, break apart the signal and determine what waves were used to make it up. (Looking back at Figure 2, the blue interference wave is made of what? Fourier transformation lets us see it has made up of the

yellow reference wave and the red analyte-sensing wave). This way, the signals can be transformed into data that can be more easily interpreted as a response due to the presence of the target analyte.

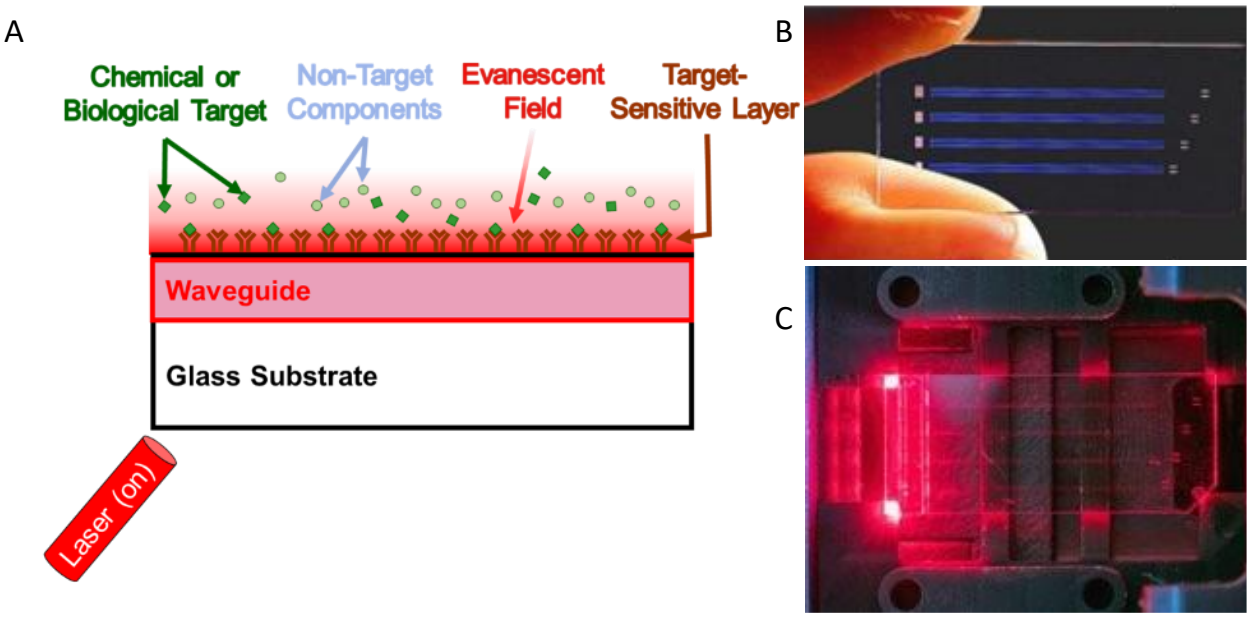

Figure 3: Salvus waveguides propagate laser light to enable detection of target analyte binding within the evanescent field **A)** Glass substrate with waveguide material coated with a target analyte-sensitive layer of receptors. As the coated waveguide is exposed to the fluid sample, target analytes create a signal as they bind within the evanescent field generated above the waveguide while the laser light source is on. The evanescent wave is propagated along the channels by the waveguide material. **B)** Layout showing input gratings on the left, four sets of two channels (one is etched and is coated in the target-sensitive receptor layer, and one is a "buried" reference), which appear blue, and staggered output gratings on the right. **C)** A visualization of the waveguide material propagating the laser light along the channels.

The presence of a chemical or biological target in the fluid media sample is measured as it is sequestered within the evanescent field generated by the light traveling through the waveguide. Sequestration occurs as the analyte interacts with a receptive, sensing molecule (a receptor) chosen to pull the target out of the fluid and onto the etched channel's surface. Receptors can be chemical or biological, such as polymers, oligonucleotides (used for their sequences of short chains of the building blocks of genetic material, DNA and RNA), proteins (antibodies, antigens, enzymes, etc.), aptamers (nucleic acid chains that are used for their structure rather than their sequence), and others. As the channels are exposed to the fluid sample, the target analytes that are present in the sample will bind to the receptor on the surface, changing the refractive index within the evanescent field that forms when the laser is on. This change in refractive index will in turn cause a phase shift in the light going through the etched channel relative to the light going through the parallel reference channel, ultimately leading to a shift in the fringes. Again, the shift can be measured and transduced to allow for mathematical analysis and generation of interpretable results.

Receptors need to be only as specific as the target requires. The Salvus assay development team will collaborate with clients to discuss their specifications and deliver the appropriately sensitive, specific, accurate assay(s). Consider the following examples.

(I) If a group is screening for a particular genetic variant in patient samples, using an appropriate oligonucleotide as the receptor will let them detect that variant and no other genetic variations.

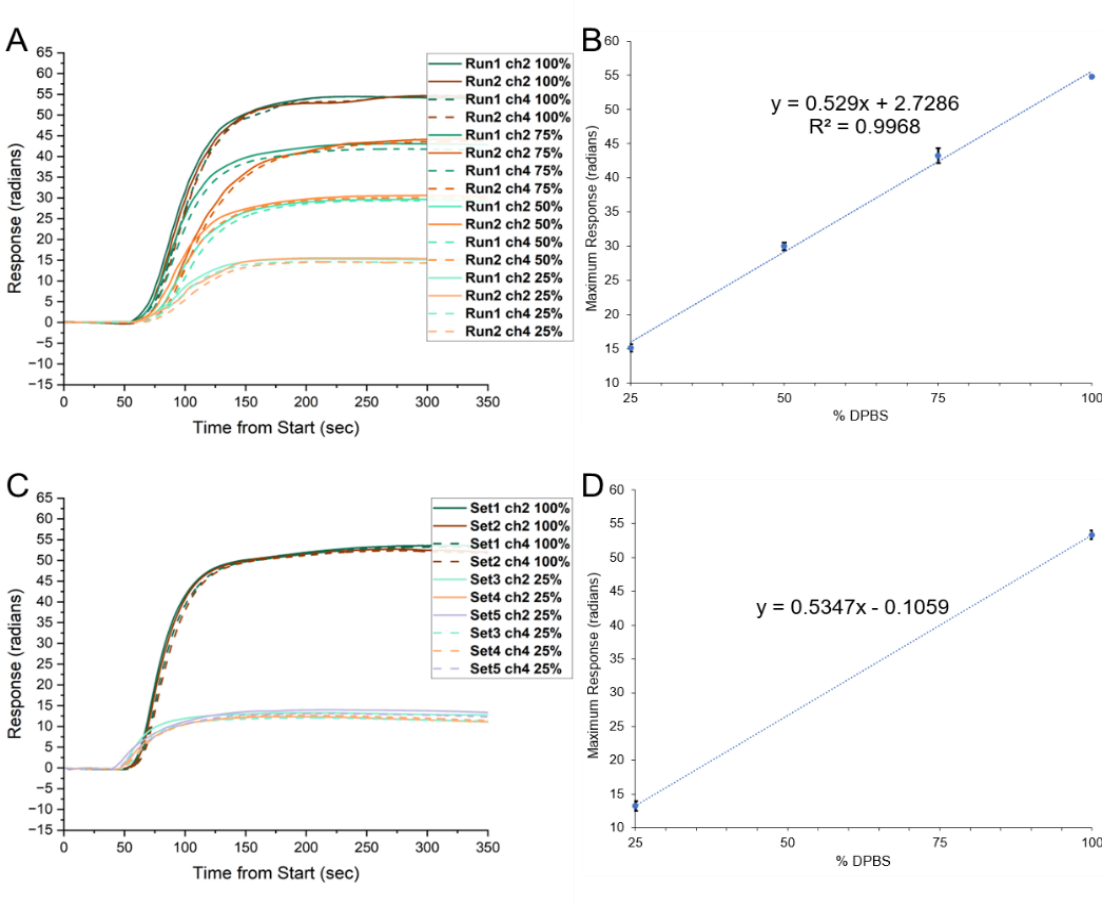

Figure 4: Technical and experimental replicates using DPBS salt solution and deionized water to generate a concentration curve

A) Technical replicates completed with 25%, 50 %, 75% and stock (100%) concentrations of a salt solution, DPBS. Deionized water was used a running buffer that was flowed through the cartridge. After reaching a baseline measurement, flow was switched to receive DPBS, and the resulting change in refractive index was measured. This experimental run was repeated with the same set of waveguides using the different concentrations. Data was collected using detection unit 1.

B) The maximum response measured from A) is plotted versus the tested % of DPBS in solution. A linear best-fit line is included with the R^2 value showing how well the data fit the line. These concentration curve data could be used to determine the percent of DPBS in solution after measuring the response of an unknown sample concentration.

C) Experimental replicates completed with either 25% DPBS salt solution on detection unit 2 or 100% DPBS salt solution on detection unit 3 with five different sets of waveguides.

D) The maximum response measured from C) is plotted versus the percent of DPBS salt solution The comparable slopes of the best-fit lines in B) and D) show that the data are consistent between running technical or experimental replicates across instruments.

(II) On the other hand, if someone only wants to measure the presence of gram-positive bacterial contamination in a filtered water sample and does not care to know what species of bacteria are there, the receptor should be broadly specific to detect all gram-positive bacteria.

These measurements can also be quantitative or qualitative as needed. Quantitative results require the generation of a concentration curve (see example data in Figure 4) that measures the detection of controlled amounts of the target to determine its mathematical relationship with the radian response detected with the technology. Qualitative results only require that the receptor be sensitive enough to detect at a desired level. And because there are currently four waveguide interferometers combined onto the glass substrate, up to three targets can be detected during a single test (the fourth is used as a control). Imagine then, if someone is trying to find out if their animals have a virus, one channel can have a receptor against that virus (broad specificity), one channel can have a receptor for a reportable strain of the virus (higher specificity), and one can have a receptor for a perhaps more common strain of the virus (higher specificity). Such a test would allow this caretaker to swiftly take the best course of action.

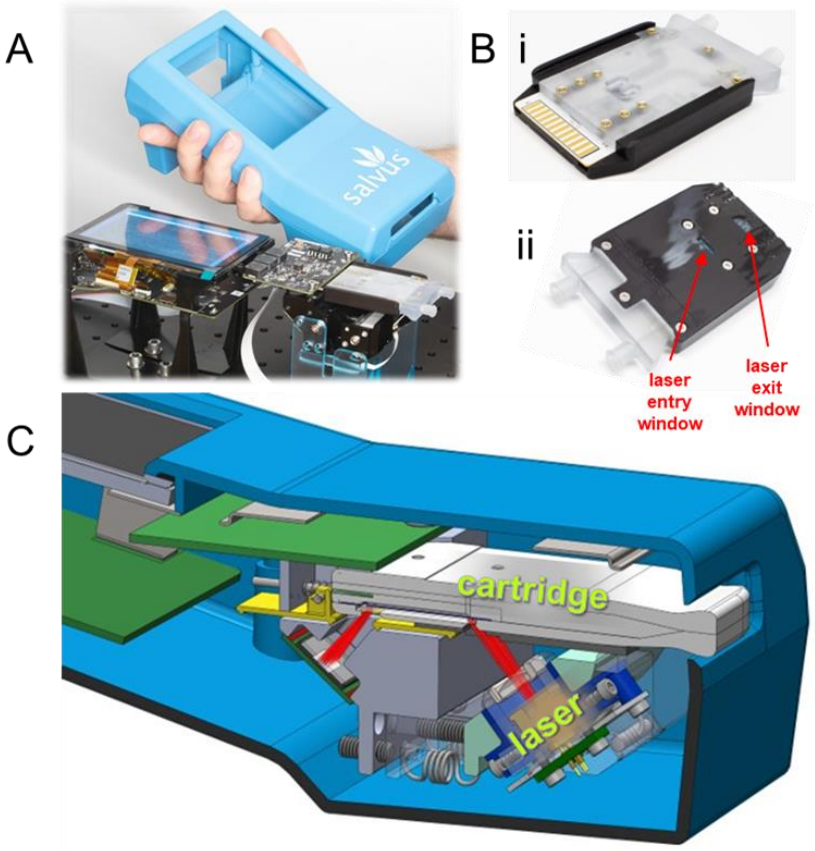

Figure 5: Cartridge positioning once inserted into sensor housing containing touch screen, laser source, camera transducer, and data communication hardware
A) The analyzer hardware is shown removed from the instrument housing but still arranged similarly to the assembled form**. B)** Top (i) and bottom(ii) views of current flow cartridge. **(i)**The top view shows the fluid flow path leading to and away from where the waveguides will sit (surrounded by the gold screws on the lower left side). **(ii)** The bottom view shows the windows through which the laser light enters and exits the cartridge to illuminate the assay channels and to display fringes for the camera to image for measurement, respectively. **C)** Cross-section of the instrument showing arrangement of the assembled hardware where the laser light meets the bottom of the inserted cartridge and exits to project onto the imaged surface.

The Salvus Benefit

The current Salvus device form factor uses a cartridge, containing the sensing chip and allowing for sample introduction, which is inserted into the analyzer encompassing the laser and transducer components (see Figure 5). The current cartridge design is a flow cartridge, for which sample fluid is held in an

external vessel and flowed over the sensor for measurement. Additionally, designs have been made for a discrete cartridge to allow for better containment, for instance, in the case of more hazardous sample types. The instrument housing also holds other parts that allow for additional convenient capabilities. The device receives power through an interchangeable, rechargeable battery or by plugging in a power adapter. It has integrated GPS tracking for those that need it. Furthermore, data transfer can occur over Bluetooth, wi-fi or ethernet.

Depending on the clients' needs, modifications could be made to the data storage, transfer, and tracking capabilities to what is required in a specific project or for a particular use. After target detection results are in, they are stored on the device until the user connects to a desired network to store or transfer the data off the device, if necessary. Presently the unit can store 32 gigabytes of data, which – contingent upon the data requirements or length of the tests – permits around fifty tests to be stored before needing to transfer or replace previous data. Also, the handheld device only weighs about two pounds (one kilogram), so carrying it with you to a point-of-use station or around several sites in the field is effortless.

The current design integrates three target assays and one control assay per cartridge. If someone needs to detect more than three targets, luckily, it is easy to swap cartridges with different assay sets to quickly evaluate another set. But that is only with the current form factor. The Salvus technology can be integrated into a variety of form factors, and the current handheld form can also be placed on the benchtop or bedside as needed. Other prospective forms include a monitoring unit that remains on-site for longer periods of time and units mounted on unmanned vehicles (see Figure 6).

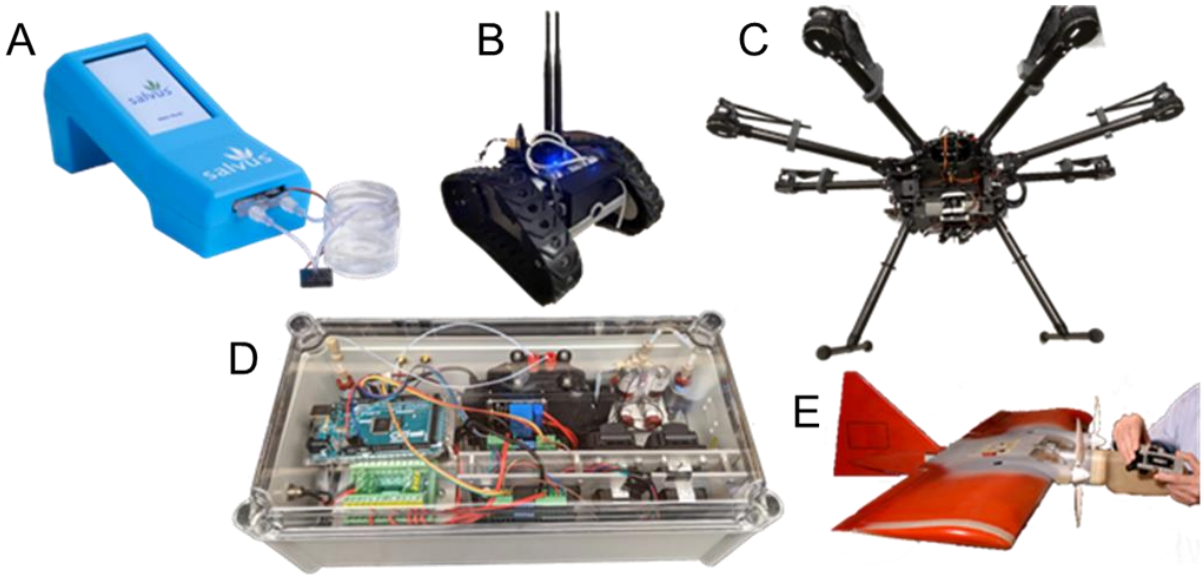

Figure 6: Form factors for various Salvus detection technology applications
A) A self-contained handheld unit. **B)** An unmanned ground vehicle with an integrated Salvus technology. **C)** An unmanned air vehicle with the technology attached to receive air samples. **D)** A monitoring device with liquid as the fluid sample. **E)** An alternative unmanned air vehicle with integrated Salvus technology able to receive air samples.

We at Salvus are dedicated to providing a high-quality detection product – the confidence, specificity, and accuracy of laboratory results while using a field-compatible device for decisions at point-of-use. We are a team of professionals with chemistry, biology, and manufacturing backgrounds, led by business experts who have been involved in various industries for over 35 years. We have licensed technology established by food processing technology experts at the Georgia Tech Research Institute (GTRI) who have worked on investigating and improving the underlying Salvus technology for more than 20 years, and we continue to collaborate and consult with them to advance the Salvus technology. With our testing process, move ahead while competitors idle – your results at hand in minutes, not weeks. Our website, salvusdetect.com, has summary information on our technology and our team of experts, as well as contact information. Contact us here at Salvus - We Detect Your Concerns™ because we believe tomorrow will be different while we keep striving to move humanity forward.

References

1. *The Economic Impact of Crop and Livestock Diseases*. Case Studies in Agricultural Biosecurity. (n.d.). https://biosecurity.fas.org/education/dualuse-agriculture/1.-agroterrorism-and-foodsafety/economic-impact-of-crop-and-livestock-diseases.html
2. Azooz, M. F., El-Wakeel, S. A., & Yousef, H. M. (2020, September). *Financial and economic analyses of the impact of cattle mastitis on the profitability of Egyptian dairy farms*. Veterinary world. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7566233/
3. Malcata, F. B., Pepler, P. T., O'Reilly, E. L., Brady, N., Eckersall, P. D., Zadoks, R. N., & Viora, L. (2020, July 30). *Point-of-care tests for bovine clinical mastitis: What do we have and what do we need?: Journal of Dairy Research*. Cambridge Core. https://www.cambridge.org/core/journals/journal-of-dairy-research/article/pointofcare-tests-for-bovine-clinical-mastitis-what-do-we-have-and-what-do-we-need/9338E0196D4055EF63D7B35A842CE993
4. *Farm nitrogen pollution damage estimated at $157 billion yearly*. Environmental Working Group. (2023, August 17). https://www.ewg.org/news-insights/news/farm-nitrogen-pollution-damage-estimated-157-billion-yearly
5. Alonzo, M., Alder, R., Clancy, L., & Fu, S. (2022, April 29). *Portable testing techniques for the analysis of Drug Materials*. Wiley Interdisciplinary Reviews. https://wires.onlinelibrary.wiley.com/doi/10.1002/wfs2.1461
6. Ph.D., J. X. (2019, June 18). *Biosensor-based Rapid Pathogen Detection: Are we there yet?* Food Safety RSS. https://www.food-safety.com/articles/6257-biosensor-based-rapid-pathogen-detection-are-we-there-yet#:~:text=Current%20levels%20of%20detection%20are%20in%20the%20femtomolar,the%20need%20for%20additional%20washing%20or%20incubation%20steps.
7. Xu, J., Suarez, D., & Gottfried, D. S. (2007). *Detection of avian influenza virus using an interferometric biosensor*. ResearchGate. https://www.researchgate.net/profile/Jie-Xu-80/publication/6130338_Detection_of_avian_influenza_virus_using_an_interferometric_biosensor/links/540eeed00cf2f2b29a3ca3c2/Detection-of-avian-influenza-virus-using-an-interferometric-biosensor.pdf
8. Xu, J., Feng, K., & Weck, M. (2011, March 5). *Free chlorine sensing using an interferometric sensor*. Sensors and Actuators B: Chemical. https://www.sciencedirect.com/science/article/abs/pii/S0925400511001663#:~:text=Chlorine%20sensing%20was%20accomplished%20by%20measuring%20the%20refractive,the%20evanescent%20field%20extended%20above%20the%20waveguide%20surface.
9. Mehrotra, P. (2016). *Biosensors and their applications - A Review*. Journal of oral biology and craniofacial research. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4862100/