WHITE PAPER

Salvus™ Detection Technology provides rapid detection of harmful and non-degradable PFAS compound PFOA using handheld device.

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Drinking water is incredibly important to everyday life and ensuring human health, requiring it to be carefully monitored with necessary regulations. Drinking water also provides a glimpse into our past where actions and processes of yesterday can persist and cause problems for the health of people today. In April 2024, the United States Environmental Protection Agency (US EPA) released a final rule setting limits on certain per- and polyfluoroalkyl substances (PFAS) compounds in U.S. drinking water. The most restrictive limits were set for perfluorooctanoic acid (PFOA) and perfluoro octane sulfonic acid (PFOS) at 4 parts per trillion (ppt)[1] (Figure 1A).

PFOA is an industrial surfactant that was first added to processes in 1947 and continued to be used in a wide range of materials from paints and lubricants to nonstick cooking surfaces until use of the chemical was halted in 2015[2, 3]. Over 60 years of production and waste resulted in PFOA contamination in the environment across the entire globe, threatening drinking water and the safety of humans on every continent, in every city and within every family.

A

Chemical	Maximum Contaminant Level Goal (MCLG)	Maximum Contaminant Level (MCL)
PFOA	0	4 ppt
PFOS	0	4 ppt
PFNA	10 ppt	10 ppt
PFHxS	10 ppt	10 ppt
HFPO-DA (GenX chemicals)	10 ppt	10 ppt
Mixture of two or more: PFNA, PFHxS, HFPO-DA, and PFBS	Hazard Index of 1	Hazard Index of 1

Maximum Contaminant Level Goal (MCLG): The level of a contaminant in drinking water below which there is no known or expected risk to health. MCLGs allow for a margin of safety and are non-enforceable public health goals. **Maximum Contaminant Level (MCL):** The highest level of a contaminant that is allowed in drinking water. MCLs are set as close to MCLGs as feasible using the best available treatment technology and taking cost into consideration. MCLs are enforceable standards. **ppt:** parts per trillion. **Hazard Index (HI):** The Hazard Index is a long-established approach that EPA regularly uses to understand health risk from a chemical mixture (i.e., exposure to multiple chemicals). The HI is made up of a sum of fractions. Each fraction compares the level of each PFAS measured in the water to the health-based water concentration.

B

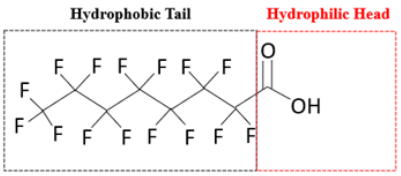

Figure 1. A) EPA limit on PFOA[1]. B) Chemical structure of PFOA.

PFOA is a member of the PFAS group of compounds. These compounds are categorized by their fluorocarbon hydrophobic tail connected to a hydrophilic functional group (Figure 1B). PFOA's hydrophilic head makes it water soluble, and the strength of the carbon-fluorine bonds gives the compound high stability and persistence in its environment. The resistance of PFOA to breakdown has given it the label of a 'forever chemical' that has dangerous impacts when present at high enough concentrations[4]. High levels of exposure can lead to certain types of cancers, is harmful during pregnancy, and can lead to changes in both cholesterol and blood pressure[5]. PFOA's persistent adsorption properties make it a dangerous risk to human health. PFOA has been found bound to human blood serum and binds through interaction with the

transport protein, human serum albumin[6]. High levels of risk caused the US EPA to create and enforce strict regulations governing low levels of PFOA in drinking water. As more studies are done on the safety of PFAS compounds and their ramifications, the US EPA could enforce even stricter limits, magnifying the critical nature of low sensitivity and rapid detection.

PFOA is not only resistant to usual environmental degradation, but it also creates difficulties for detection and removal processes due to its persistence. At such low allowed limits (parts per trillion level), water samples are typically tested in centralized laboratories far away from the contaminated sources. Centralized lab testing has resulted in added cost and time delays for those managing the problem. Salvus has set out to make PFOA detection quick and reliable by focusing on three technological advancements.

- Commercialization of a portable handheld device that can rapidly perform tests in about 15 minutes at any site with possible PFOA contamination.
- Optimization of a polymer, PFOA detecting sensor that uses interferometry to deliver repeatable, low levels of detection.
- Development of a better process that more accurately measures low levels of PFOA at the source that would otherwise go undetected.

Developing a Rapid Detection Solution

The development of the innovative PFOA assay and sensing unit combines Salvus' high standards for detection while meeting practical, commercial use requirements. Imagine that at an industrial site, PFOA-containing products are disposed of improperly, resulting in PFOA leeching into nearby waters either through direct spillage or ground water run-off. This area is likely part of a much larger ecosystem and watershed that feeds into ground water and eventually drinking water. Even if the disposal and possible contamination is considered an issue immediately, samples need to be collected, processed, and sent off to a laboratory for analytical testing. Now it's weeks later and the localized spill has become a widespread health hazard putting public health at risk. Salvus' rapid handheld device brings analytical sensitivity and standards to the location in need of testing, saving both time and money in testing and downline remediation.

PFOA is just one of the many PFAS compounds that are regulated by EPA and can cause health concerns to the public. The Salvus technology has been optimized and developed using PFOA as the main analyte of interest, with application to adapt the assay to additional PFAS compounds. To achieve repeatable, low-level detection of a target analyte using a sensor, there are important properties to consider.

- What is the binding mode of the analyte to be detected?
- What ligand has the proper interaction capabilities to bind to the analyte of interest, and how can it be immobilized to the sensing surface?
- What buffer allows for favorable binding of the analyte to the sensing surface?
- How complex is the matrix that the analyte is in, and how can any effects from this be minimized?

Salvus detection technology uses waveguides to provide a path for light waves to travel inside the waveguide. The light that travels through the waveguide material also results in a light wave oscillating over the sensing surface, forming what is described as the evanescent field (Figure 2A). The evanescent field will be able to pick up the local refractive index changes caused by the chemical bindings between the ligand and analyte with the highest degree of sensitivity using interferometric measurement configuration. The detection sensitivity is directly related to the ratio of evanescent field intensity to the total propagated light. As you move further away from the evanescent field with your ligand, the ratio of light intensity decreases, therefore decreasing the sensitivity capabilities of the sensing surface (Figure 2B). Patterned channels on the waveguide surface provide a guided location for immobilization (Figure 2C). Within these channels, ligand receptors can be placed by coating the channel. This allows for measurement within the evanescent field and allows Salvus to coat individual channels independently to provide multiplexed detection capabilities. Additionally, a reference channel is implemented for quality control during tests.

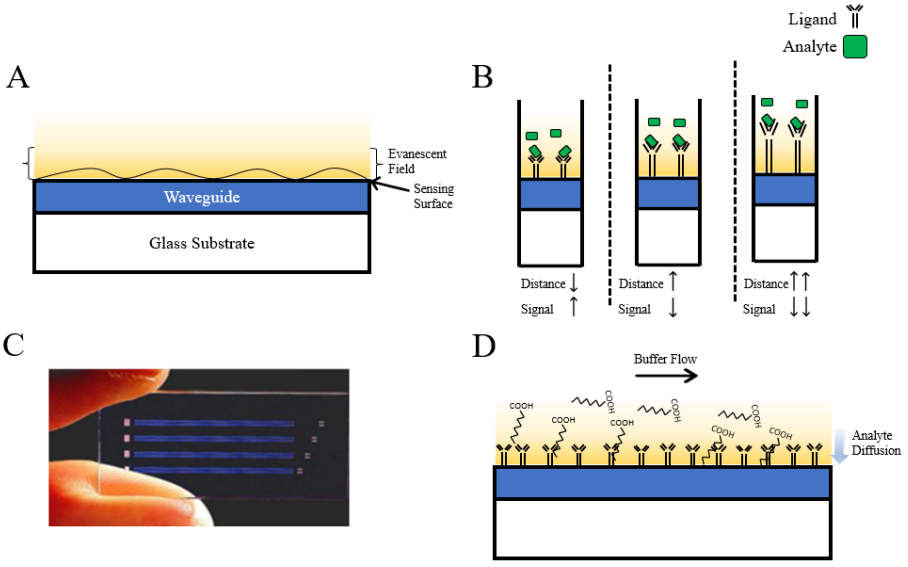

Figure 2. A) Waveguide design including evanescent field. B) Schematic of relationship between receptor location in evanescent field to signal. C) Image of Salvus waveguide used for PFOA detection assays. D) Schematic of PFOA binding to sensing surface receptors.

Currently, non-covalent interaction between the receptor and PFOA is used to bind PFOA with specificity for the hydrophobic tail (Figure 2D). The Salvus team focuses on sensitivity and specificity requirements for all ligands based on regulatory requirements and customer needs. This includes tuning the ligand receptor to specifically detect all PFAS compounds rather than having the focus on just one. The polymer chemistry was first designed by Georgia Tech Research Institute (GTRI) to be used in PFOA detection. GTRI has over two decades of experience investigating and optimizing the technological foundation used in this detection and continues to be a source of consultation about the device. Salvus, with a licensing agreement for this technology, has focused on increasing the efficiency of the process as well as the optimization and validation of a repeatable and sensitive coating layer.

Detection of PFOA Using Salvus Technology

The lightweight, handheld Salvus device utilizes an easy-to-use, load-and-remove cartridge that contains the sensing waveguide and allows for the easy and mess-free addition of the sample, keeping any potential hazardous materials away from the user and keeping the sample running over the waveguide. Waveguides are coated with the PFOA sensing film, and the surface is washed with buffer for equilibration and activation of the surface.

Buffer consideration for PFOA detection is crucial to ensure the analyte will not only dissolve in solution, but also promote the interactions between the receptor and PFOA. Salvus has guided buffer development and choice based on not only assay and analyte compatibility, but also aligning buffer choice with factors that include being:

- A viable, safe commercialized product
- Compatible with buffers that may be required during the sample analysis process of PFOA from samples of interests

Once the waveguide has been exposed to the optimized buffer, PFOA samples are injected into the Salvus unit in the same buffer. Sample injection results in a change in the fringe pattern due to the binding of PFOA to the ligand and causes a change in refractive index at the sensing surface layer. This change results in a binding curve over time that will tell you the presence of PFOA and what concentration of PFOA is in your sample in as little as 10 minutes from sample injection. Once the analyte detection results are obtained, a screen displays if PFOA has been detected and the value to use for concentration determination (Figure 3). The data is stored within a 32-gigabyte drive in the unit that can be transferred to a desired network. This allows enough storage to do 50 PFOA tests before they need to be transferred and stored elsewhere.

Figure 3. Salvus analyzer after completed PFOA detection run.

During the sample, PFOA reaches the waveguide, diffuses from the buffer toward the sensing layer, and binds to the ligand. In the evanescent sensing layer, it is reporting association response of the analyte to the ligand with a direct relationship to analyte concentration (Figure 4A). Obtaining the response at given concentrations can be used to construct a standard or calibration curve that can identify the concentration of unknown field samples that fit within the curve's confines. Laboratory-made samples of various concentrations of PFOA were made in 100% ethanol to run as the sample, with ethanol to be used as the equilibration running buffer. Stock PFOA in ethanol was diluted to the following concentrations, in parts per billion (ppb), to construct the standard

curve: 0, 100, 250, 500, and 750 ppb. The point at which the running buffer is switched to PFOA sample buffer is the 0-time mark on the graph shown (Figure 4B.) At around 100 seconds (about 1.5 minutes) after switching to sample, the sample begins to reach the waveguide and sensing layer (Figure 4A, B). The sample continues flowing over the sensing layer for 600 seconds (about 10 minutes) after the initial switch. At the 600-second point, injection is switched back to the 100% ethanol running buffer.

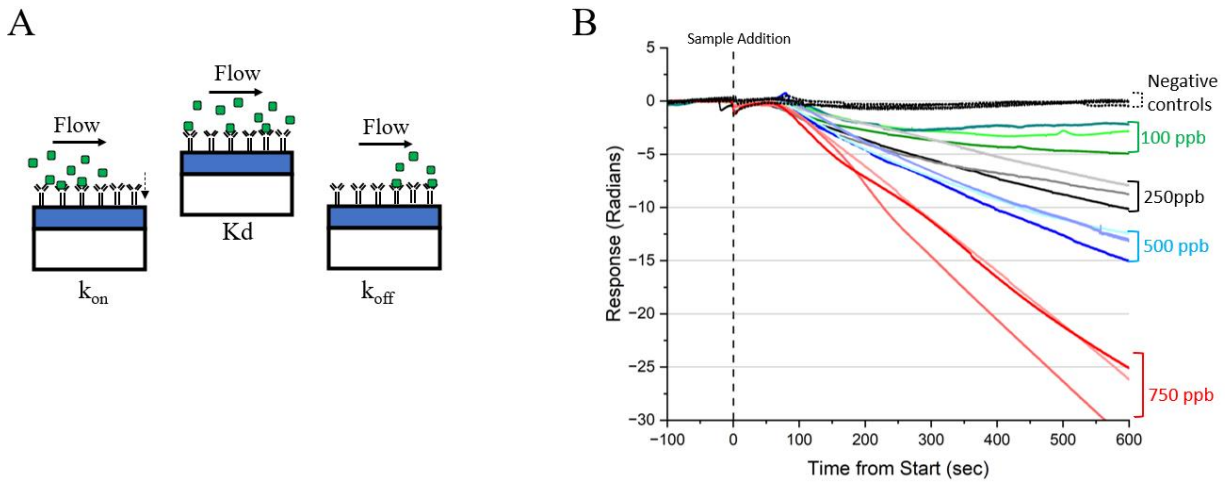

Figure 4. A) Schematics depicting analyte binding at each step of sample addition. B) Concentration Summary Graph demonstrating range and response for a given concentration of PFOA. Blanks are included to show response is a result from analyte binding to receptor.

The response of different PFOA concentrations can be observed by the slope of the curve after sample initially hits the sensing layer, or from total response of the sensing layer (max-min during sample window). This information is useful for the end user as the test will not only show that PFOA has been detected, but also relate the data to a known standard curve to identify the contamination levels. Our current detection demonstrates the ability to detect concentrations from 100 ppb to 750 ppb quantitatively as shown in our summary graph while also showing how the response compares to the negative controls (Figure 4B).

A set of standard response curves, Figure 4B, gives valuable analysis tools right in the customer's hand, and it can be customized to fit the need of the customer. If the customer wishes to know the exact concentration, then Salvus can provide them with the data to achieve that. And for customers whose main concern is understanding if their levels of PFOA are within an acceptable range, they may prefer a simpler result that states if the levels are at an allowable level or not. Future decreasing of our level of detection and further validating our standards curves will aid in the optimization and development of all areas of the PFOA sensing assay, including building on the regeneration ability of the sensor. It will also give us the ability to provide the customer with a test for PFOA that can be used multiple times, saving costs that are not available in a single-use test.

PFOA, like all PFAS compounds, is not going away any time soon. The more we learn about PFOA, the more we realize it is a real problem that requires real solutions, and quickly. An earlier example described an industrial site producing products with PFOA that had improperly disposed of materials and led to possibly high PFOA levels. In that scenario, they sent the samples to a lab and waited two to three weeks for results, depending on potential delays. During that time, the contaminated water went from a localized spill to a drinking water catastrophe. Instead of sending samples to a lab and waiting two to three weeks, the Salvus handheld unit tests samples at the point-of-concern and delivers the result in minutes instead of weeks. This reduction in time means reduction in both risk and dollars spent.

Adaptable, deployable, and sensitive, our Salvus Detection Platform is an advanced solution that delivers results within minutes in the field so users can make quicker decisions and initiate more timely action. At Salvus, we're focused on providing innovative, user-friendly detection solutions that advance humanity by empowering informed decisions. Our team has expertise in the fields of chemistry, biology, manufacturing, and entrepreneurship with experience ranging from agriculture, chemical formulation, assay development, and other industrial processes. Contact us to discuss how we can customize a solution for you. We Detect Your Concerns™.

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