JSTO in the News

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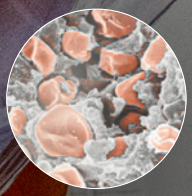
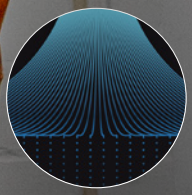
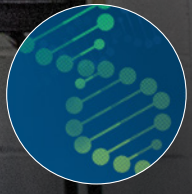


The Race Against Resistance



Frugal Filters, Flawless Performance



Synthetic Molecules

DTRA MISSION

DTRA provides cross-cutting solutions to enable the Department of Defense, the United States Government, and international partners to Deter strategic attack against the United States and its allies; Prevent, reduce, and counter Weapons of Mass Destruction (WMD) and emerging threats; and Prevail against WMD-armed adversaries in crisis and conflict.

CHEMICAL AND BIOLOGICAL TECHNOLOGIES DEPARTMENT MISSION

Lead DoD science and technology to enable the Joint Force, nation, and our allies to anticipate, safeguard, and defend against chemical and biological threats.

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Front cover: U.S. Army SSGT Mauricio Caceres, survey team member, 21st Weapons of Mass Destruction-Civil Support Team (21st WMD-CST), New Jersey National Guard, operates a particle extractor during an Army North mandated training proficiency evaluation. The particle extractor removes aerosols and transfers them to a container for later testing. The 21st WMD-CST supports civil authorities at man-made or natural disasters by identifying chemical, biological, radiological, and nuclear substances, assessing the consequences, and advising on response measures. (New Jersey National Guard photo by Mark C. Olsen)

Inside cover: U.S. Air Force SSGT Renee Delgado, 18th Medical Group lab technician, applies an antibiotic to a blood plate. The lab is meant to provide doctors with more accurate diagnostic information than what can be determined through basic symptoms. (U.S. Air Force photo by Senior Airman Quay Drawdy)

Back cover: Recruits with U.S. Marine Corps Papa Company, 2nd Recruit Training Battalion, complete the gas chamber phase during chemical, biological, radiological, and nuclear defense training. Recruits are exposed to CS (tear) gas to familiarize themselves with the use of their gas masks. (USMC photo by LCpl Ava Alegria)

THE RACE AGAINST RESISTANCE

Biothreats persist in their pursuit; developing new antibiotics is a way to *stay ahead*.

Researchers are developing new antibiotics to combat common bacteria that are becoming increasingly resistant to antibiotic treatment, which is a growing concern for both public health and Joint Force readiness. Both emerging pathogens and known biothreats, such as *Bacillus anthracis* (anthrax), *pestis* (plague), *Francisella (F.) tularensis* (tularemia, also called "rabbit fever"), *Burkholderia mallei* (glanders), and *Burkholderia pseudomallei* (melioidosis), are all capable of antibiotic resistance—whether naturally occurring or deliberately engineered by an adversary.

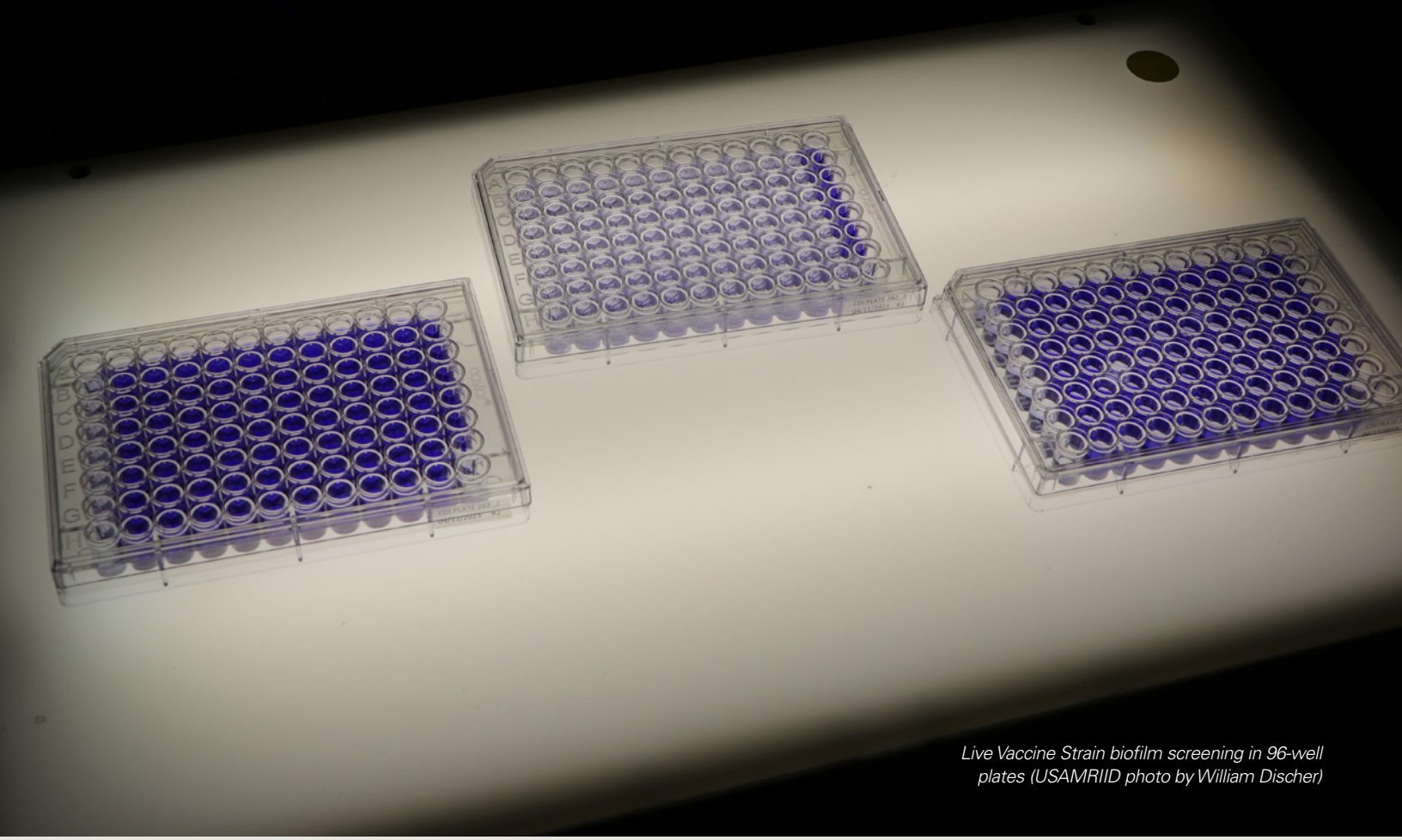
To help deter the threat of antibiotic resistance in these pathogens, the Defense Threat Reduction Agency's (DTRA) Chemical and Biological Technologies Department in its role as the Joint Science and Technology Office (JSTO) for Chemical and Biological Defense, an integral component of the Chemical and Biological Defense Program, partnered with the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) and Walter Reed Army Institute of Research to identify targets for developing new antibiotics.

Recently, USAMRIID investigators collaborated in screening chemical compounds for potential use against *F. tularensis*, the cause of tularemia in humans. Tularemia is typically carried by rodents and rabbits in the Northern Hemisphere and can spread to humans through contact with infected animal tissues, deer flies, and ticks. While the overall number of human tularemia cases is small (about 300 per year in the United States), the bacterium is designated by the Department of Health and Human Services and the U.S. Department of Agriculture as a Tier 1 select agent, "presenting the greatest risk of deliberate misuse with most significant potential for mass casualties or devastating effects to the economy, critical infrastructure, or public confidence," because of its low infectious dose (as few as 10–15 organisms), high morbidity, and ease of aerosolized inoculation, which make it a potential biological weapon.

"…there is a *need* to identify new therapeutics to combat tularemia in the event resistant *F. tularensis* isolates or variants threaten the Joint Force."

Current antibiotics such as fluoroquinolones (Cipro and Avelox) and aminoglycosides (Gentamicin and Streptomycin) are effective for treating tularemia; however, high levels of resistance have been increasingly observed for this and other gram-negative pathogens, and there is a well-documented ability to derive resistant *Francisella* strains. Given these conditions, there is a need to identify new therapeutics to combat tularemia in the event resistant *F. tularensis* isolates or variants threaten the Joint Force.

USAMRIID's team implemented a two-pronged approach for identifying antibacterial compounds to combat antibiotic resistance in *F. tularensis* (and potentially other bacterial biothreats and public health pathogens).



Live Vaccine Strain biofilm screening in 96-well plates (USAMRIID photo by William Discher)

The first approach is to examine targets of *F. tularensis* that are critical to the bacteria and then exploit these targets to identify new inhibitors. Current laboratory efforts focus on three structures of the bacterium: biofilm, peptidoglycan, and lipopolysaccharide.

The second approach is to cast a broader net to identify new inhibitors. USAMRIID has a procedure for high-throughput screening of compounds able to block the growth of Live Vaccine Strain—a version of *F. tularensis* that can be safely handled in a Biosafety Level 2 laboratory. So far, the team has screened nearly 50,000 compounds from an exploratory chemical library.

Several compounds are currently being characterized to determine if inhibition occurs with the fully virulent bacteria (in a Biosafety Level 3 laboratory) to ensure that surrogate screening can be recapitulated in relevant strains. Other studies are underway to assess the suitability of these compounds for further development as antibiotics, and to determine whether they could be used against additional bacterial threat agents.

The next step for this drug discovery process is to test the lead compounds from the library for their ability to protect against *F. tularensis* in a challenge model. The successful lead candidates will be considered for advanced development and future clinical trials so that DTRA JSTO can prevail against *F. tularensis* and other similar and future biological threats. ●

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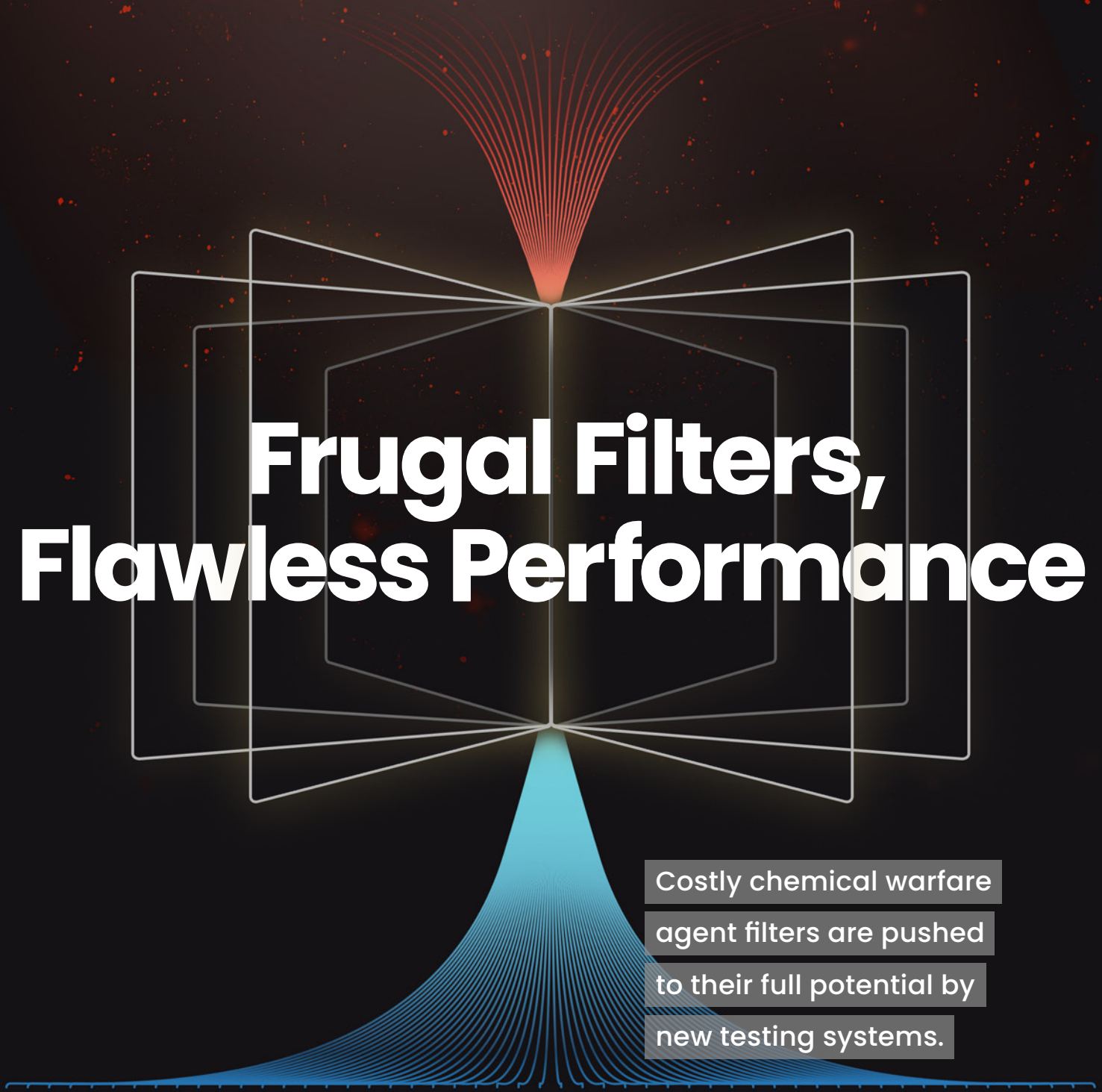
Frugal Filters, Flawless Performance



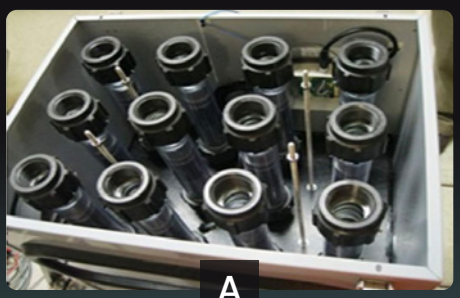
Costly chemical warfare agent filters are pushed to their full potential by new testing systems.

A new Residual Life Indicator (RLI) and Sampling Cartridge Analysis System (SCAS), currently in development, will enable the Department of Defense (DoD) to less expensively maintain collective protection (ColPro) systems in buildings and ships. These ColPro systems use large, expensive filters that operate 24/7 to provide uncontaminated air in the event of a chemical warfare agent attack.

Filters must be changed on a regular maintenance schedule to ensure that their protective capacity is not depleted. The current method for testing remaining filter capacity includes disassembling and testing the filters in a laboratory, which destroys them. The RLI and SCAS will allow DoD to monitor ColPro filters life in real time and change them only when filter capacity is depleted, without destroying expensive filters for testing.

A nondestructive, onsite testing method would enable an operator to measure the remaining service life of usable filters and replace them only when needed, which would minimize life-cycle expenses.

Over time, pollution and humidity reduce filter protective capacity, even in the absence of chemical warfare agents. These ColPro filters must be replaced on a regular maintenance schedule that includes a costly verification of system integrity. Replacing filters on a regular schedule minimizes the risk to the Joint Force but results in higher life-cycle costs for ColPro systems. Ideally, filters should only be replaced when their protective capacity is depleted. A nondestructive, onsite testing method would enable an operator to measure the remaining service life of usable filters and replace them only when needed, which would minimize life-cycle expenses.



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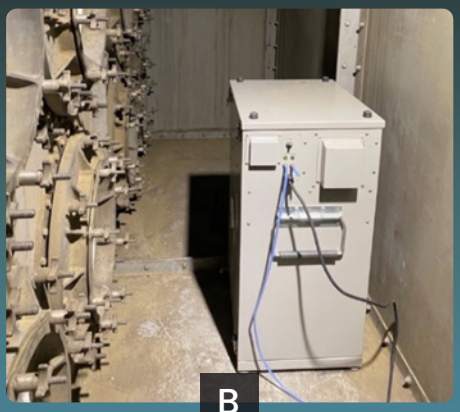
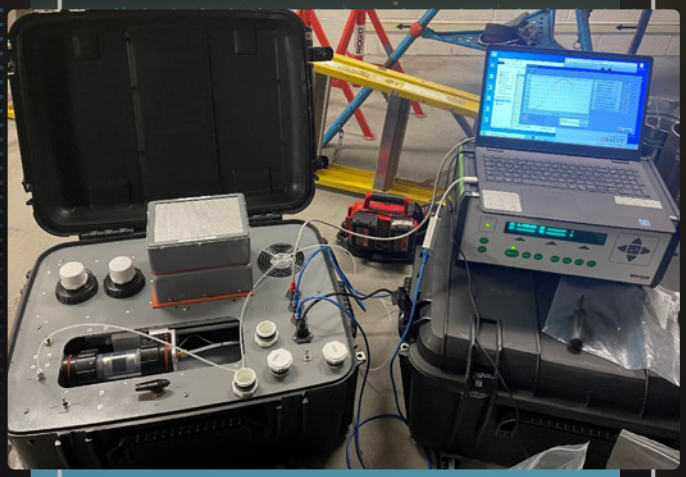


B

(A) Residual Life Indicator (RLI) cartridges are housed in the cartridge sampling system that holds up to a dozen cartridges. (B) The RLI cartridge sampling system is installed near a bank of ColPro system filters. (DEVCOM CBC photos)

A single RLI cartridge. (DEVCOM CBC photo)

To develop a nondestructive, onsite testing method for fixed-site and shipboard ColPro filters, the Defense Threat Reduction Agency's (DTRA) Chemical and Biological Technologies Department in its role as the Joint Science and Technology Office (JSTO) for Chemical and Biological Defense, an integral component of the Chemical and Biological Defense Program, is collaborating with the Combat Capabilities Development Command Chemical and Biological Center (DEVCOM CBC) to develop RLI-SCAS. DEVCOM CBC began to develop the RLI-SCAS in 2016 as a two-part system.



A small RLI cartridge in the slot being tested in the Sampling Cartridge Analysis System. (DEVCOM CBC photo)

The first RLI part consists of a small filter indicator cartridge housed in a manifold and installed in line with the fixed-site or shipboard ColPro system. The indicator cartridge is a miniature filter containing the same type of materials as the fixed-site or shipboard ColPro filter. RLIs are housed in a manifold called a cartridge sampling system that holds up to a dozen cartridges. As the fixed-site or shipboard ColPro system draws external air, the same air is drawn into the RLI cartridge sampling system. At scheduled maintenance intervals, individual RLI cartridges are pulled from the system and tested for remaining capacity. Because the cartridges are exposed to the same environment as the fixed-site or shipboard ColPro system, RLI cartridge capacity reflects remaining filter capacity. Between 2016 and 2020, DEVCOM CBC performed multiple fixed-site and shipboard technology demonstrations to ensure that RLI cartridges accurately predict the remaining ColPro system filter life.

The second part of the system is the SCAS. SCAS is a portable instrument the size of several suitcases. It enables onsite, nondestructive analysis of remaining RLI cartridge capacity. A technician with the SCAS could visit the fixed site or ship at designated maintenance intervals and test RLI cartridges only. If testing indicated that RLI cartridge capacity remained above specified values, the filters would not be changed. However, if testing indicated that RLI cartridge capacity was depleted, the filters would be replaced. In this manner, the two-part RLI-SCAS enables customized replacement schedules of expensive filters without increasing risk to the Joint Force and reduces ColPro system life-cycle costs.

From 2018 to 2023, DEVCOM CBC demonstrated the RLI-SCAS in multiple DoD buildings and ships worldwide and collected real-world data for system performance. The data from shipboard demonstrations allowed the researchers to optimize the technology to ensure that RLIs accurately predicted ColPro filter capacity in all operationally relevant environments. Data from fixed-site demonstrations showed that RLI cartridges accurately reflected large ColPro filter residual capacity over 39 months with 95% confidence. Ongoing demonstrations at fixed sites will be completed in early FY24 and the RLI-SCAS technology will transition to the Modernization Collective Protection program of record near the beginning of FY25.

In the future, all DoD fixed-site buildings and ships with ColPro systems may use an RLI cartridge sampling system to monitor remaining filter capacity and optimize maintenance and change-out schedules based on environment. Technicians with SCAS may routinely visit these sites and ships, test the RLIs, and change the filter only when necessary. This will reduce the time and life-cycle costs maintaining ColPro systems without impacting the risk to the Joint Force. ●

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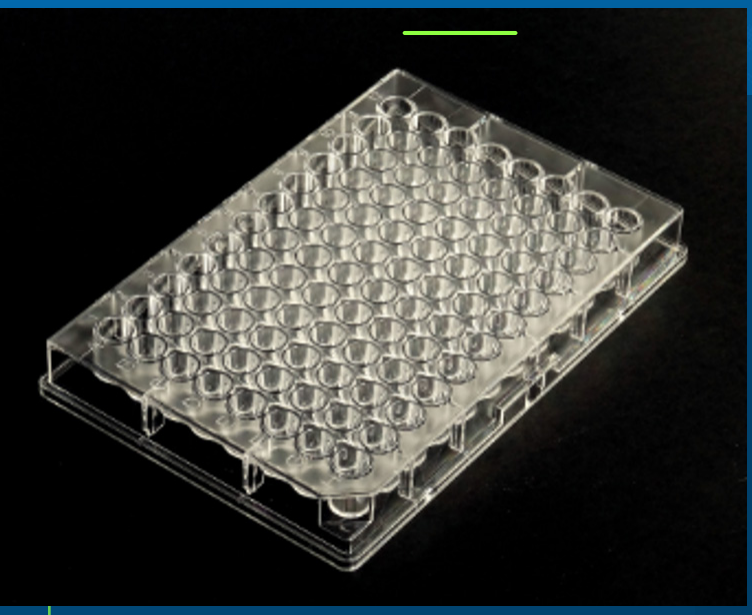
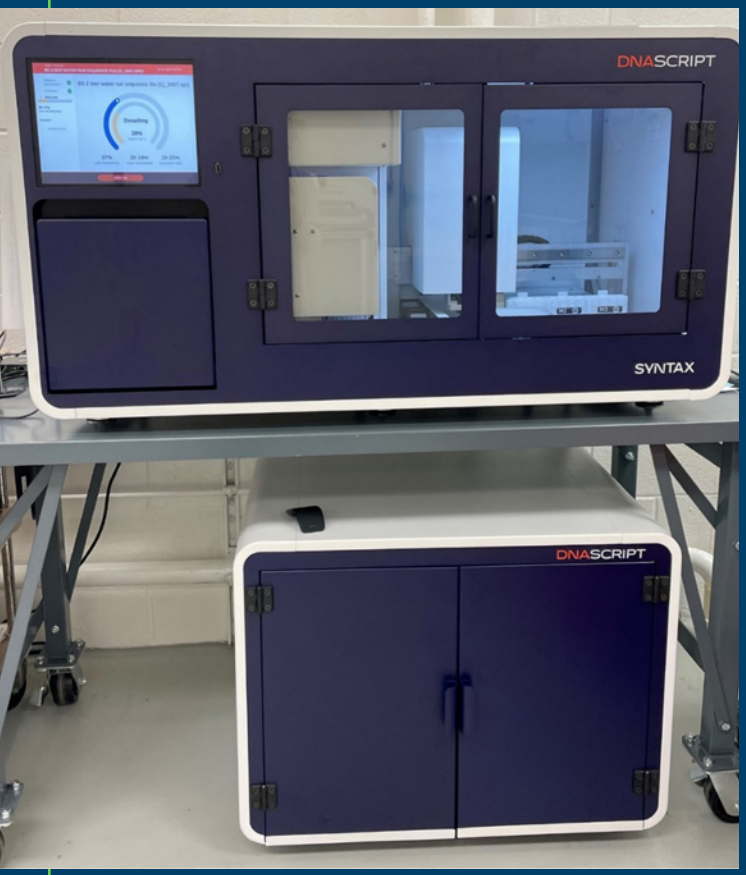
Synthetic Molecules

Benchmarking the platforms to improve DNA synthesis.

Advancements in DNA synthesis technologies have the potential to lower some of the technical and costly barriers that scientists have faced since the discovery of the DNA molecular function. DNA has become a valuable tool for forensics, chemical, and biological sciences capable of generating biological systems, including organisms, from synthetic genomes. Synthetic biology is an area where technological advances affect the threat landscape that will ultimately influence chemical and biological (CB) defense to protect the warfighter from current and emerging CB threats. These new advancements include creating synthetic DNA faster.



Example of a 96-well plate. (Biomat photo)

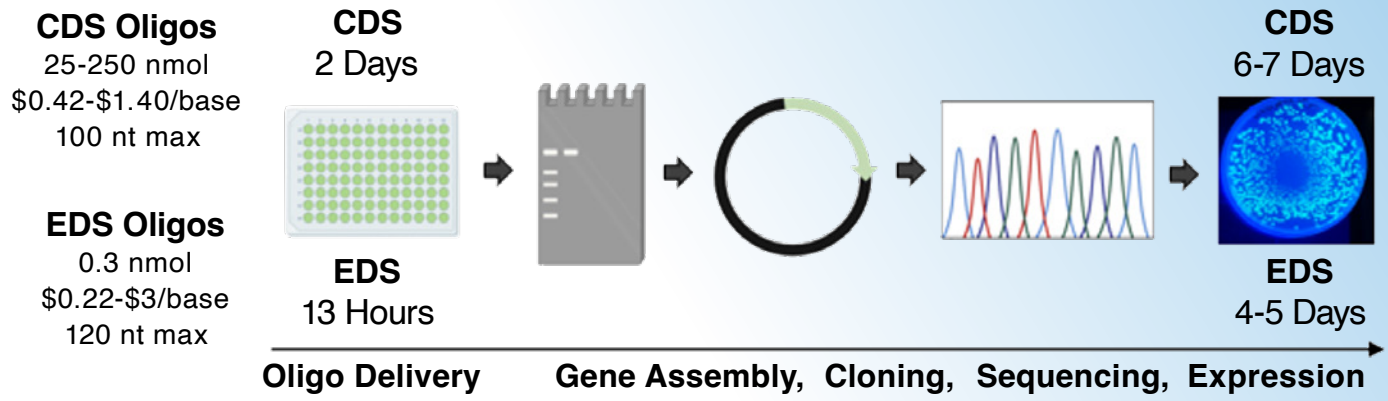


Syntax-200 Benchtop Enzymatic DNA Synthesizer (DEVCOM CBC photo)

To take advantage of the significant advancements in DNA synthesis that have occurred over the past five to ten years, the Defense Threat Reduction Agency's (DTRA) Chemical and Biological Technologies Department in its role as the Joint Science and Technologies Office (JSTO) for Chemical and Biological Defense, an integral component of the Chemical and Biological Defense Program, invested with the U.S. Army Combat Capabilities Development Command Chemical Biological Center (DEVCOM CBC) to assess new enzymatic methodologies in terms of technical advantages, costs benefits, and an overall decrease of technical barriers.

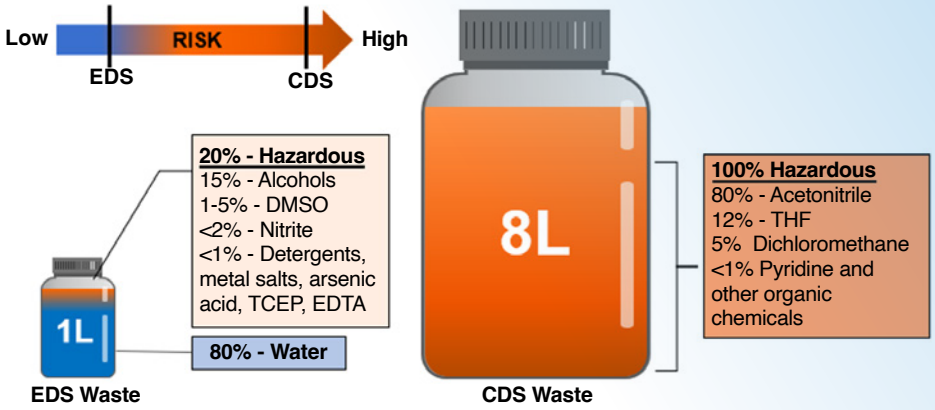
The DEVCOM CBC team evaluated commercial enzymatic DNA synthesis (EDS) printers as they offer a user-friendly platform and predominantly aqueous waste stream compared to the traditional large amounts of waste generated by chemical DNA synthesis (CDS). Scientists showed that a benchtop EDS can produce a 96-well plate of 60-nucleotide-long oligonucleotides ("oligos") in 13 hours, including cleanup and quantitation (the average concentrations of DNA present in a mixture, as well as their purity). In contrast, an equivalent run on a benchtop CDS platform requires two days for synthesis, cleanup, and quantitation. Commercial oligos then typically require a two-day delivery. Notably, the EDS instrument produced only 1 liter of primarily aqueous, non-hazardous waste compared to 8 liters of hazardous organic waste for a similar run on a benchtop CDS platform.

The researchers also evaluated enzymatically produced oligos alongside chemically produced commercial oligos to assemble a synthetic gene-encoding Green Fluorescent Protein (GFP). The EDS platform produced lower concentrations of individual oligos, but these were available in half the time of commercially produced oligos and were sufficient to assemble functional GFP sequences of comparable accuracy without producing hazardous chemical waste.



(Top) Methodology used to compare 120 nucleotide-maximum oligos produced enzymatically (Syntax-100, DNA Script, Inc.) to chemically synthesized 100 nucleotide-maximum oligos (IDT) in gene assembly. (DEVCOM CBC image)

(Right) Comparison of waste produced from benchtop enzymatic DNA synthesizer (EDS) with chemical DNA synthesizer (CDS) for production of 96 oligos, 60 nucleotides long. (DEVCOM CBC image)



"Overall, benchtop EDS platforms can produce more oligos in a shorter time frame at a comparable cost to similar CDS instruments..."

Currently, phosphoramidite-based CDS is the standard method for creating new single-strand DNA building blocks and is used extensively by commercial vendors. The advances in EDS make this technology competitive with chemical methods, with the potential of shifting to large-scale enzymatic DNA synthesis in the near future.

Overall, benchtop EDS platforms can produce more oligos in a shorter time frame at a comparable cost to similar CDS instruments while offering a convenient, user-friendly interface with control over final yield, sequence-editing capabilities, and an environmentally friendly waste stream. The knowledge and experience required to use these benchtop enzymatic DNA platforms is minimal and significantly lowers the barriers for use across many disciplines and applications. However, only one company currently sells a benchtop EDS instrument, which increases the overall cost of the instruments and reagents. Additionally, the current benchtop EDS platform is less amenable to manual user input throughout the synthesis compared to CDS instruments and requires skilled personnel to address instrument malfunctions or errors. Nevertheless, multiple companies have made strides in using EDS methods to overcome the 100-oligo length limitation faced by chemical methods by producing single oligos up to 1000 nucleotides long.

With such advances, enzymatically produced DNA methods will continue to evolve, substantially changing the biological threat landscape with the production of longer DNA sequences for future applications to protect the Joint Force. ●

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2024 CBD S&T Conference

SAVE THE DATE

December 2-5, 2024

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The 2024 CBD S&T Conference — hosted by the Defense Threat Reduction Agency's (DTRA) Chemical and Biological Technologies Department in its role as the Joint Science and Technology Office (JSTO), an integral component of the Chemical and Biological Defense Program — brings together scientists, researchers, program managers, and military personnel to defend our nation and its warfighters against the most pressing chem-bio threats of tomorrow. Hear about the latest groundbreaking research.

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CBD S&T CONFERENCE 2024

Within the Defense Threat Reduction Agency's Research and Development Directorate resides the Chemical and Biological Technologies Department performing the role of Joint Science and Technology Office for Chemical and Biological Defense, an integral component of the Chemical and Biological Defense Program. This publication highlights the department's advancements in protecting the Joint Force, our nation, and allies from chemical and biological threats through the innovative application of science and technology.

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