Manipulating a flask of cells for live virus testing

Human Trials Show Counter-Ebola Promise
Early Detection Stops Diseases
CB Sensors: To Detect and Protect
Microbial Kill Switches Target Toxins
Lead DoD science and technology to anticipate, defend and safeguard against chemical and biological threats for the warfighter and the nation.

On the cover:
A Centers for Disease Control microbiologist manipulates a flask of cells used in experiments with live viruses. The researcher is working within the confines of an enclosed flow hood, which creates a negatively-pressurized environment which prevents air from within the hood from re-entering the laboratory area.
Last year’s Ebola crisis in West Africa killed 11,000 people and infected more than 28,000, highlighting the need for urgent and effective medical countermeasures to fight the disease.

The virus’s high lethality also makes it a cause for concern as a biological weapon against our nation and warfighters, especially those who are stationed in Africa.

Fortunately, a new drug compound to combat the Ebola virus was successful in its Phase I human trials, giving hope that the drug can advance as a potential countermeasure.

During the Single Ascending Dose study, conducted by the Defense Threat Reduction Agency’s Joint Science and Technology Office and the U.S. Army Medical Research Institute of Infectious Diseases, the new compound GS-5734, manufactured by Gilead Sciences, demonstrated no adverse events.

The next test, scheduled for early 2016, will continue the human safety studies in Phase Ib, the Multiple Ascending Dose study. If the compound continues to demonstrate no adverse events, the U.S. Department of Defense and Gilead will seek FDA approval, which could happen as early as 2017.

Effective countermeasures must be able to work both prior to and post exposure, be manufactured quickly in emergency surge situations, and gain regulatory approval from the U.S. Food and Drug Administration. To do this, the DoD leverages internal expertise in the biological threat agents and pharmaceutical industry.

Prior to the human trials, GS-5734, was tested in non-human primates and demonstrated 100 percent protection, even when administered up to three days after infection.

During the course of testing, several potential candidates were identified by JSTO for review; however, GS-5734 demonstrated highly desirable qualities for a drug candidate.

This compound is part of the polymerase inhibitor family. Because polymerase enzymes are responsible for replicating the virus in the body, inhibiting the replication is an effective way to stop the virus. GS-5734 and similar compounds trick the virus into thinking they are correct building blocks of the disease, but when the virus attempts to incorporate them, replication is halted.

GS-5734 is a promising compound and JSTO will continue to explore its applicability to the warfighter against viral threats. In this way, JSTO remains focused on providing medical countermeasures that are safe and effective against biological threats to protect the warfighter.
A host of illnesses threatens people year round—from colds, to the flu, to stomach ailments. While the average person can take a few days off to recover from an illness, warfighters engaged in combat cannot. They must continue to execute their mission, regardless of their ailment, making an accurate diagnosis and treatment critical to mission success. Is the warfighter experiencing the flu, or have they been exposed to a chemical or biological agent that presents similar symptoms? These questions need answering as quickly and accurately as possible in order to provide the best treatment options.

In a project managed by Dr. Nathan Adams from the Defense Threat Reduction Agency’s Joint Science and Technology Office, BioFire Diagnostics is moving their popular FilmArray Respiratory Panel from licensed clinical laboratories to locations with greater utility for military troops such as clinics, ships or aid stations. There, health care workers can run one test that will identify one of 14 common pathogens within an hour.

From influenza, arainfluenza, the common cold, and whooping cough to coronavirus, the virus that caused severe acute respiratory syndrome, health care workers will be able to identify and treat a sick warfighter quickly and accurately.

However, JSTO and BioFire Diagnostics envision a more beneficial use of the FilmArray and will begin working with the U.S. Food and Drug Administration to develop a biowarfare agent panel, named WARRIOR.

WARRIOR can likewise be used at the point of care, rather than requiring samples go back to certified labs for testing. This allows for faster identification and treatment of an exposed warfighter. Following that strategy, patients sick from biowarfare agents such as anthrax, plague, Ebola and Marburg viruses can be isolated within an hour in order to quickly contain the outbreak.

While WARRIOR is undergoing development and may take several years to gain FDA approval, military health care workers continue to gain experience with the FilmArray Respiratory Panel.

Since WARRIOR will mimic the current FilmArray Respiratory Panel, health care workers can seamlessly integrate WARRIOR once FDA approval is granted. FilmArray and WARRIOR will be tied into the Global Information Grid, the U.S. Department of Defense’s end-to-end system for warfighters, support personnel and government leaders.

This capability will allow commanders to assess outbreaks in real time. As these point of care diagnostics see greater use, disease outbreaks will stop in their tracks, due to more effective diagnosis, treatment and isolation, protecting both warfighters and the general population.
Engineering at the molecular scale, particularly the design of active nanosystems, has resulted in advances in protecting the warfighter, particularly in the area of chemical and biological agents. These efforts improve understanding and afford enhanced fabrication and performance characteristics for new materials that have applications in detection, decontamination, protection and elimination of chemical and biological threats.

The Directed Transport Materials (DTM) basic research program, managed by the Defense Threat Reduction Agency’s chemical and biological technologies manager Dr. Brian Pate, provides important information and capabilities that utilize surface directed molecular transport techniques to separate and concentrate chemical and biological simulants. The results from the DTM program enable new approaches in a wide range of nanotechnologies, including biosensing, drug delivery, molecular assembly and active materials. Recent research and development efforts resulted in new intellectual property filings and patent awards for novel directed transport material systems and analytical methods capable of characterizing these systems at the molecular level.

As a result, the team obtained two provisional patents, “Ultra-sensitive in situ fluorescence analysis using modulated fluorescence interference contrast at nanostructured surfaces” and “Fast single molecule localizations by pattern matching.” These efforts will significantly improve speed, sensitivity and specificity of molecular detectors and assays.

Another project within the DTM program focuses on developing enzymatic nanoscale chemical pumps that automatically turn on when they detect a signal from a chemical or biological agent, thereby enabling the development of novel detection platforms. These materials will be capable of removing contamination and serve as self-decontaminating materials. The DTM team successfully met key milestones and filed a patent titled “Self-Powered Enzyme Micropumps.” This research team is continuing to develop and refine two proof of concepts for these enzyme pumps. One will detect and destroy organophosphate based nerve agents and the other will detect anthrax lethal factor and generate a sterile region around the pump.

These complementary theoretical and experimental research efforts are advancing U.S. defense capabilities in areas such as super-resolution localization microscopy, single-molecule tracking, modeling concepts for directed transport applications, and accelerated analyte capture and detection. These advances will ultimately enable new device applications that combine sensing and microfluidic pumping into single self-powered microdevices for new cost effective chemical and biological detection and decontamination capabilities, protecting the warfighter at home and abroad.

Researchers in the DTM program have successfully met a major milestone by developing and validating an iterative algorithm tool that estimates the position and intensity of a single fluorophore. This result has revolutionized the way molecular interactions are probed, the environments where this is possible, and the resolution that can be achieved by use of light microscopy for a single molecule.

Enzyme pumps are enabled by the fluid motion of either the substrate or related biomarker. Image courtesy of principal investigator Dr. Ayusman Sen, Pennsylvania State University.

POC: Dr. Brian Pate; brian.d.pate.civ@mail.mil
Scientists at the Massachusetts Institute of Technology developed two genetic biocontainment systems, or kill switches called ‘deadman’ and ‘passcode,’ to limit the viability of the host microbe to a specific environment based on the presence of small molecules. With widespread use of genetically engineered microbes in military, medical and industrial applications, it is increasingly important to develop these security measures to protect our warfighters. These kill switches prevent the release of hazardous substances to public environments and thwart the theft of these valuable properties by adversaries.

Current biocontainment systems use auxotrophic microbes by removing genes to generate cellular dependency on an exogenously supplied metabolite for survival or by altering translational codon usage to necessitate unnatural amino acids for protein production. However, this approach is limited and subject to the availability of the metabolite or the unnatural amino acid. Additionally, production of auxotrophic microbes may require extensive genetic modification, which limits their application.

Cells containing the ‘deadman’ switch must stay in an environment with the presence of an exogenously supplied chemical, anhydrotetracycline (ATc), to block toxin expression. Since ATc is a synthetic compound that does not exist in a natural environment, engineered cells are unlikely to receive this signal for proliferation upon escaping from their assigned environments. Additionally, the LacI inducer Isopropyl β-D-1-thiogalactopyranoside may be used to independently activate the circuit to induce cell death even in the presence of ATc. Thus, target cell survival can be controlled by two different operator inputs.

The team developed a second biocontainment system that can respond to a broad range of potential environmental cues by establishing a set of hybrid transcription factors (TFs). These hybrid TFs are composed of DNA recognition modules (DRMs) that bind to specific DNA operator sites, and environmental sensing modules (ESM) that respond to the presence of specific small molecules to control binding affinity between the DRM and operator.
By mixing and matching these DRMs and ESMs, the team constructed hybrid TFs that enable connections of different small molecule input to the control of a specific promoter for gene expression. In the resulting series of ‘Passcode’ circuits, these hybrid TFs are used to create logic-gate behavior in which multiple inputs are used to regulate the same promoter. By incorporating these hybrid circuits into a two-layered transcription design, the authors establish a set of ‘Passcode’ circuits that allow cell survival under only one of the eight potential combinations. Importantly, the survival signal combination can be easily reprogrammed by rewiring the input sensing to promoter activity.

Dr. Collins’ team used the ‘deadman’ and ‘passcode’ circuits to control a range of toxic genes including the cytotoxic endonuclease EcoRI and a mf-Lon protease-mediated degradation system. The combination of EcoRI expression and mf-Lon protease-mediated essential protein MurC degradation provided the strongest biocontainment protection, with cell survival rates dropping more than a million-fold in just six hours of exposure to non-survival conditions.

The team found that the killing efficiency of both the ‘deadman’ and ‘passcode’ circuits gradually diminished over time, predominately caused by mutations in the toxin genes. To address this problem, the team removed the mutagenic elements from the E. coli genome, which improved the ‘passcode’ cell killing rate by three to five orders of magnitude after four days, suggesting that the robustness of circuit-based biocontainment systems can be elevated by increasing the host genetic stability.

Dr. Collins and his team believe that these circuit-based biocontainment systems will be useful to the armed forces, biotechnology and pharmaceutical industries as they can be used to confine engineered cells to highly specific environments. Finally, these kill switches protect the warfighter by ensuring biotoxins, if obtained by our adversaries, are not used against friendly forces.

To read more about the study, find the Nature Chemical Biology article, “‘Deadman’ and ‘Passcode’ microbial kill switches for bacterial containment” recently published in Nature Chemical Biology.
Within the Defense Threat Reduction Agency’s Research and Development Directorate, resides the Joint Science and Technology Office for Chemical and Biological Defense. This publication highlights the organization’s accomplishments to protect warfighters and citizens through the innovative application of science and technology research.