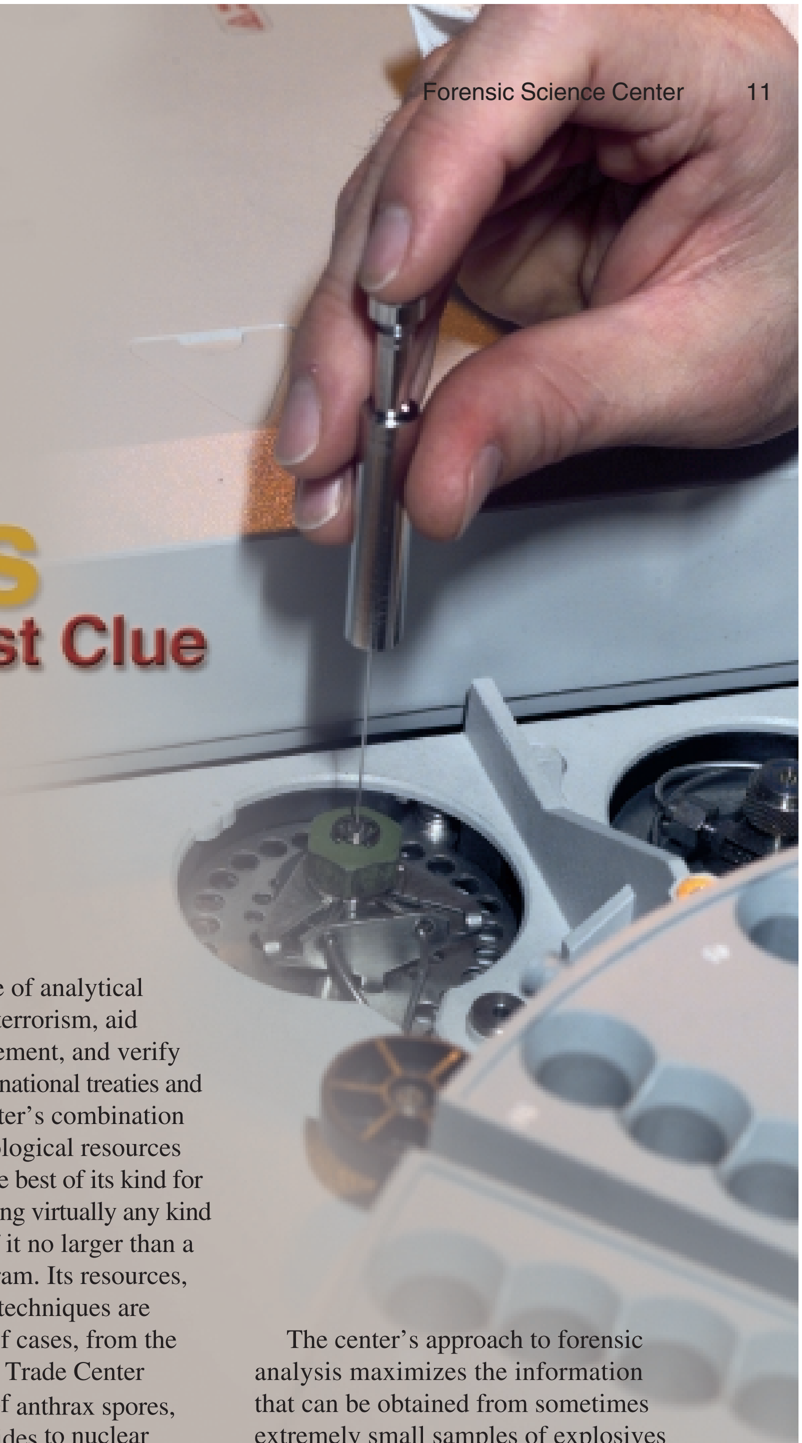
Forensic Science Center Maximizes the Tiniest Clue



Livermore chemists are coaxing a wealth of information from increasingly small samples.

WHILE Lawrence Livermore's national security accomplishments have received much publicity over the years, one Laboratory organization has gained such a stellar reputation among law enforcement, intelligence, and emergency response agencies that it is cited by Tom Clancy in his novel *Shadow Watch* (Berkley Books, 1999):

"I've requested assistance from the Forensic Science Center in San Francisco. It's at the Lawrence Livermore National Laboratory. I don't know if you're familiar with them."

"They did evidence analysis on the Unabomber case, the Times Square and WTC bombings in New York, probably hundreds of other investigations," Nimec said. "Uplink's had a relationship with them for years, and I've worked with them personally. The LLNL's the best group of crime detection and national security experts in the business."

Founded in 1991, the Laboratory's Forensic Science Center (FSC) offers a comprehensive range of analytical expertise to counter terrorism, aid domestic law enforcement, and verify compliance with international treaties and agreements. The center's combination of human and technological resources has made it among the best of its kind for collecting and analyzing virtually any kind of evidence, some of it no larger than a few billionths of a gram. Its resources, expertise, tools, and techniques are applied to all kinds of cases, from the September 11 World Trade Center attack to the spread of anthrax spores, from multiple homicides to nuclear materials smuggling.

FSC has a staff of 15 personnel, mostly chemists, with expertise in analytical chemistry, organic chemistry, inorganic chemistry, nuclear chemistry, toxicology, pharmacology, special coatings, and forensic instrument design and fabrication. The center also draws upon the resources of experts in Livermore's Chemistry and Materials Science and Nonproliferation, Arms Control, and International Security directorates.

The center's approach to forensic analysis maximizes the information that can be obtained from sometimes extremely small samples of explosives residue, dust particles, hair strands, blood stains, radioactive isotopes, drugs, chemicals, and clothing fibers. As Brian Andresen, until recently FSC director, says, "We're probing the lower limits of detection for many types of compounds isolated during an investigation." Even the tiniest quantities, says Andresen, are usually enough to provide compelling evidence that holds up in court. The minuscule amounts of oils remaining on fingerprints, for example, can tell the general age of suspects, their diet, and whether they smoke. In that respect, says Andresen, "Everything someone does leaves a chemical or biological signature that we can investigate."

Many forensic research projects have required FSC personnel to develop new analytical tools, forensic techniques for analyzing trace amounts of evidence, and unique sampling procedures. Several new, portable instruments have been developed that are capable of detailed analysis in the field. These tools provide important advantages when dealing with substances that may be unstable, perishable, or too toxic to bring back to the Laboratory.

Heather King and David Chambers demonstrate Livermore's solid-phase microextraction (SPME) sampling technique for identifying and quantifying the chemical composition of physical evidence.

Supporting International Security

Andresen notes that the term "forensic science" used to apply only to the scientific analysis of evidence for civil or criminal law. Increasingly, however, forensic analyses done at FSC are broadening that definition to include support for monitoring or verifying compliance with international treaties and agreements, particularly those involving weapons of mass destruction, and for countering threats of terrorism. For example, the center is contributing to the National Nuclear Security Administration's (NNSA's) Chemical and Biological National Security Program to develop and field advanced technologies to better prepare for, detect, and respond to chemical or biological incidents in the U.S.

In light of its demonstrated capabilities to analyze minute specimens, FSC was selected by the U.S. State Department in 2000 to support the Organization for the Prohibition of Chemical Weapons (OPCW) as the second U.S. certification laboratory. (The other facility is the Edgewood Chemical and Biological Analytical Center in Maryland.)

OPCW, based in the Netherlands, is responsible for implementing the Chemical Weapons Convention, which bans the production, stockpiling, or use of such weapons as nerve agents and blister agents. OPCW-designated laboratories test samples collected by OPCW inspectors from sources around the world to determine whether the samples contain chemical weapon agents, their precursor chemicals, or decomposition products. The convention stipulates that all samples must be analyzed at the two OPCW-designated laboratories. Federal legislation requires that all samples taken from a U.S. facility be tested in a U.S. laboratory that is OPCW-certified.

FSC has established a separate chemical weapons analysis laboratory that is certified by the American Association for Laboratory Accreditation. To date, no actual samples have been officially collected from any site or analyzed at any laboratory. FSC, however, has been required to analyze and identify constituents of mock samples supplied by the OPCW as part of a series of proficiency tests.

According to FSC's Armando Alcaraz, "Passing the tests is a very challenging task because the samples might contain literally thousands of chemicals that are linked to chemical weapons manufacturing." He notes that the samples are sometimes spiked with certain materials to deliberately try to throw the analysis teams off track. Like the test samples, the real samples will be extremely dilute (that is, parts-per-million level) so that they can be shipped commercially or sent through the mail.

Scientists from the Forensic Science Center have miniaturized thin-layer chromatography to make it suitable for field use. The portable system includes appropriate reagents, glass plates, a digital camera, and a notebook computer.

Helping Law Enforcement

FSC also assists law enforcement agencies with special needs that cannot be handled by standard crime laboratories. "We're not in the business of routine police lab work," Andresen cautions. However, for cases that are particularly difficult, FSC may be a valuable resource capable of providing a conclusive analysis. In this respect, law enforcement agencies benefit from Livermore technologies that were developed initially to support counterterrorism efforts, detect nuclear proliferation activities, and advance stockpile stewardship.

Under the 1998 "Partnership for a Safer America" memorandum of understanding between the Department of Energy and the departments of Justice, Commerce, and Treasury, the center provides law enforcement agencies such as the Federal Bureau of Investigation (FBI), the U.S. Customs Service, and the Bureau of Alcohol, Tobacco, and Firearms with new crime-fighting technologies. This agreement provides a framework for formal working relationships to facilitate the transfer of DOE technology and technical expertise to law enforcement.

FSC deputy director Pat Grant notes that supporting law enforcement increases the center's expertise and shortens the turnaround times for sample analysis. "Anytime we analyze questioned samples important to a real-world investigation, we are honing our skills. It's a much more interesting and stimulating experience than participating in an exercise."

Shrinking Instruments

Some of the center's most enduring accomplishments are new tools it has developed for intelligence, law enforcement, and health professionals working in the field. These compact, battery-powered tools provide mobile chemistry laboratories. Because they eliminate the need to ship samples back to a standard laboratory for analysis, the portable technologies greatly speed decision making.

For example, FSC scientists have miniaturized and modernized thin-layer chromatography (TLC), a well-established laboratory procedure that identifies compounds belonging to the same general chemical class. FSC chemists made TLC technology suitable for field use with a portable system that fits inside a suitcase and weighs about 23 kilograms. Although the portable system uses minimal equipment and chemical reagents, it is highly specific and sensitive. The kits can be used to analyze two sets of samples simultaneously, with each set containing about 10 samples. Depending on the compounds being analyzed for, the entire process takes 10 to 20 minutes to complete.

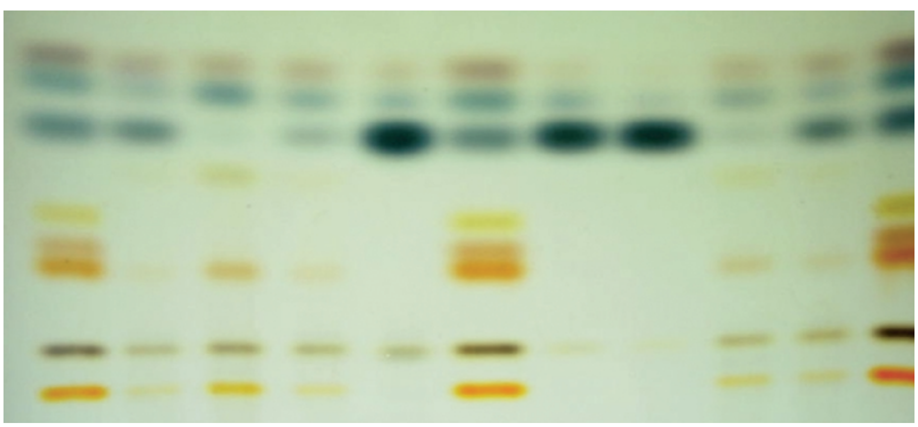
TLC works by separating compounds over the distance they move up a glass plate. Tiny amounts of samples are placed just above the bottom edge of a TLC plate, the plate is placed in a small solvent reservoir, and the solvent moves up the plate by capillary action. A commercial digital camera captures the resulting patterns of dark spots that develop, which are analyzed on a notebook computer using a software program originally developed for the analysis of DNA. Based on the distance the samples have traveled, together with their color and intensity, the computer program identifies the compounds and their relative concentrations.

The center's portable TLC kits are tailored to detect chemicals indicative of chemical weapons, high explosives, propellant stabilizers, or illegal drugs. Each specialized kit includes solvents and developing reagents that are specific to the compounds of interest.

The TLC system was originally developed for the U.S. Army to quickly detect propellant instabilities within the nation's munition storage depots. Propellants (especially high explosives) require stabilizers to prevent them from spontaneously igniting. Because stabilizers are depleted by long exposure to environmental conditions, the Army needed a way to quickly determine the safety of large numbers of munitions. The center's TLC system requires only 50-milligram samples of explosive, instead of the gram quantities typically required by other methods, and 15 minutes for each group of 20 samples, allowing many more samples to be analyzed and at much lower cost than is possible using traditional methods. "Army personnel without a degree or extensive training in chemistry can do this work," says FSC chemist Jeff Haas.

Over a few days in 1998, the portable system successfully characterized the contents of more than 1,200 unearthed mortar rounds discovered in a shallow excavation site at an Army base in Massachusetts. (See *S&TR*, December 1998, pp. 21–23.) The system is now deployed at several other Army facilities as well as by National Guard units.

The system is also used in instances where analysis speed is essential. In light of repeated success by a variety of users, the center is transferring the portable TLC technology to private industry for commercialization and widespread availability to federal and state law enforcement, customs, and environmental agencies.



The portable thin-layer chromatography (TLC) system separates compounds as they move up a glass plate placed in a small solvent reservoir. Based on the distance the compounds travel, together with their color and intensity, a computer identifies the compounds and their relative concentrations. Above is a typical TLC analysis done to detect propellant instabilities by measuring the amount of stabilizer compounds.

Advanced Tools for Field Use

While TLC is effective for identifying classes of chemicals that are specifically targeted, the task of completely characterizing samples in the field requires a more sophisticated instrument such as the gas chromatograph–mass spectrometer (GC–MS). An essential tool in every major analytical laboratory, a GC–MS can detect ultratrace quantities of organic compounds weighing a billionth of a gram or less. The gas chromatograph first slowly heats a sample to about 250°C. As the sample's volatile constituents travel down a long capillary column, they separate according to their vapor pressures and chemical affinities. As they flow into the mass spectrometer, the compounds are bombarded with an electron beam that fragments molecules into ions that constitute a unique fingerprint of that compound for positive identification.

FSC staff scientists have shrunk the standard 114-kilogram laboratory GC–MS to about 28 kilograms; it now fits inside a wheeled suitcase. The self-contained portable device, comparable in sensitivity and selectivity to a standard unit, contains a power generator, vacuum pumps, and laptop computer. The result is an instrument that significantly improves on-scene investigation and evidence collection.

Because of its ability to analyze samples to parts-per-billion sensitivity within 15 minutes, this portable GC–MS can be used to support nonproliferation activities, incident response, and law enforcement investigations. For example, the instrument can precisely identify compounds indicative of the manufacture of chemical warfare agents and illicit drugs. The instrument is currently being manufactured under license to industry.

Identification with Lasers

Although many tools used by FSC personnel depend on analyzing tiny amounts of chemicals that are found in a vapor phase above a liquid or some solid materials, most solid objects, such as human hair or clothing, do not have a significant vapor pressure and thus do not lend themselves easily to GC–MS analysis. However, center personnel can vaporize these solid samples with an extremely fine laser beam to generate wisps of product that contain identifying compounds.

The technology is called imaging laser-ablation mass spectroscopy. The process combines a laser for vaporizing extremely small amounts of material, an ion trap and time-of-flight mass spectrometer for analysis, and a high-powered microscope for viewing. In this way, forensic scientists can collect and rapidly identify suspect chemicals.

The process can be used on almost any solid material—dirt, pieces of glass, paint chips, clothing fibers, strands of hair. The samples are placed inside an ion trap mass spectrometer, irradiated with a laser, and identified within a few minutes by the mass spectrometer. The process allows an investigator to "walk down" a hair shaft by drilling consecutive holes on the same hair with the laser and analyzing each volatile sample. "Because hair grows at a standard rate, the results can reveal a history of drug use or exposure to compounds used in biological or chemical weapons manufacturing," says FSC chemist Greg Klunder. He points out that the method could also be applied to samples of clothing or soil sticking to the shoes of someone suspected of developing chemical weapons.

A similar instrument still under development is capable of detecting chemicals in air and is well suited for high-speed aircraft sampling of exhaust smoke from chemical facilities. Potential applications include identifying hazardous spills, monitoring industrial stacks for certain compounds, and surveying the environment from a remote location to detect chemical releases from a suspect facility.

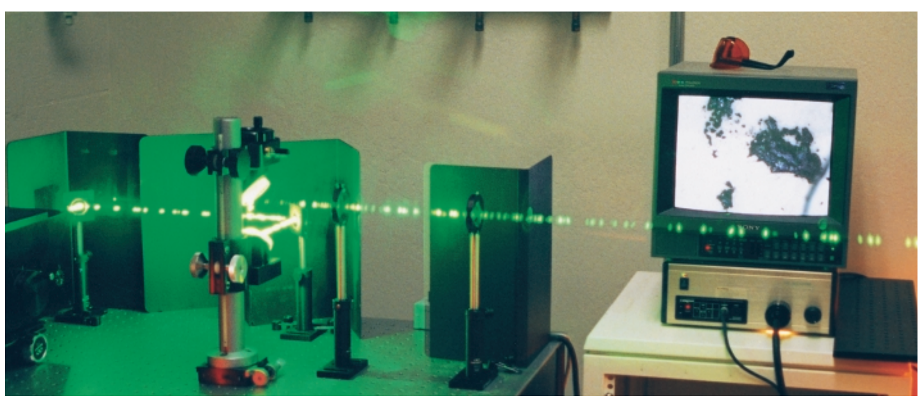
Wands of Collection

One of the center's most important developments has been the solid-phase microextraction (SPME) collection kits that use optical fibers as "chemical dipsticks" for safe and efficient sampling. "The technique has revolutionized the collection of forensic samples in the field," says FSC chemist Pete Nunes.

The technology uses commercial hair-size (100-micrometer-thick) fibers to capture organic vapors. The fiber, residing inside a syringe, is coated with a chemical polymer that, when exposed to the ambient environment for a suitable amount of time, can collect thousands of different compounds by acting as a chemical sponge. The polymer coatings are specific for different types of compounds such as chemical warfare agents, high explosives, or illegal drugs.

The collection technique requires no solvents, sample workup, or additional equipment typically associated with obtaining evidence. The fibers can be inserted directly into a portable or stationary GC–MS for immediate analysis.

Forensic Science Center chemist Del Eckels uses the 28-kilogram portable gas chromatograph–mass spectrometer that fits inside a wheeled suitcase. The portable unit, comparable in sensitivity and selectivity to much larger and heavier units, permits fast on-the-scene chemical analysis.



The imaging laser-ablation mass spectrometer combines a laser for vaporizing extremely small amounts of material, an ion trap time-of-flight mass spectrometer for analysis, and a high-powered microscope for viewing.

Nunes says that because the fibers are fragile, they had never been taken into the field. To overcome their fragility, an FSC team developed rugged aluminum transport tubes, with each tube securing one syringe and fiber. A group of five tubes is contained in each kit. The hermetically sealed tubes prevent any possibility of cross-contamination and support chain-of-custody requirements. A sampling port in the bottom of the tube permits assaying the contents in a glove box before the tube is actually opened.

SPME sampling is being put to good use by FSC weapons scientist David Chambers to monitor nuclear weapon warheads safely. This activity is part of the NNSA's Stockpile Stewardship Program to maintain the safety and reliability of the nation's nuclear stockpile.

Center Plays Role in Famous Law Enforcement Cases

The Forensic Science Center (FSC) has played a pivotal role in several well-publicized criminal investigations. For example, FSC examined the composition and structure of tiny bomb fragments containing trace metal and chemical residues in the Unabomber case.

The center provided analysis and testimony leading to the conviction of Fremont, California, bomber Rodney Blach, a former Chicago Police Department forensic investigator. Blach was convicted of planting bombs during 1998 at the homes of the police chief, a city council member, and others. Former FSC Director Brian Andresen helped investigators from the federal Bureau of Alcohol, Tobacco and Firearms (ATF) to reconstruct what Tom Rogers, assistant district attorney, characterized as "the largest as well as the most electronically sophisticated domestic pipe bombs the ATF had ever encountered." Rogers said, "The electronic aspects of the devices were beyond the expertise of anyone at the ATF."

FSC supported the Democratic National Convention in 2000 by providing a mobile forensic laboratory for the Los Angeles County Sheriff's Terrorist Early Warning Group. The center was also instrumental in interpreting factors surrounding the death of Gloria Ramirez, who made several hospital emergency room personnel violently ill in a well-publicized Southern California case.

FSC helped prosecutors in Glendale, California, rearrest Efren Saldivar, the self-proclaimed Angel of Death and alleged killer of many terminally ill hospital patients. FSC scientists performed toxicology analyses on exhumed tissues from 20 patients. They didn't expect to find anything. However, with the help of completely new techniques, including sample collection procedures developed by the center, they were able to identify the drug Pavulon in the bodies of six of the deceased patients. The rearrest of Saldivar was based primarily on the center's findings

Identifying Bullet Fragments

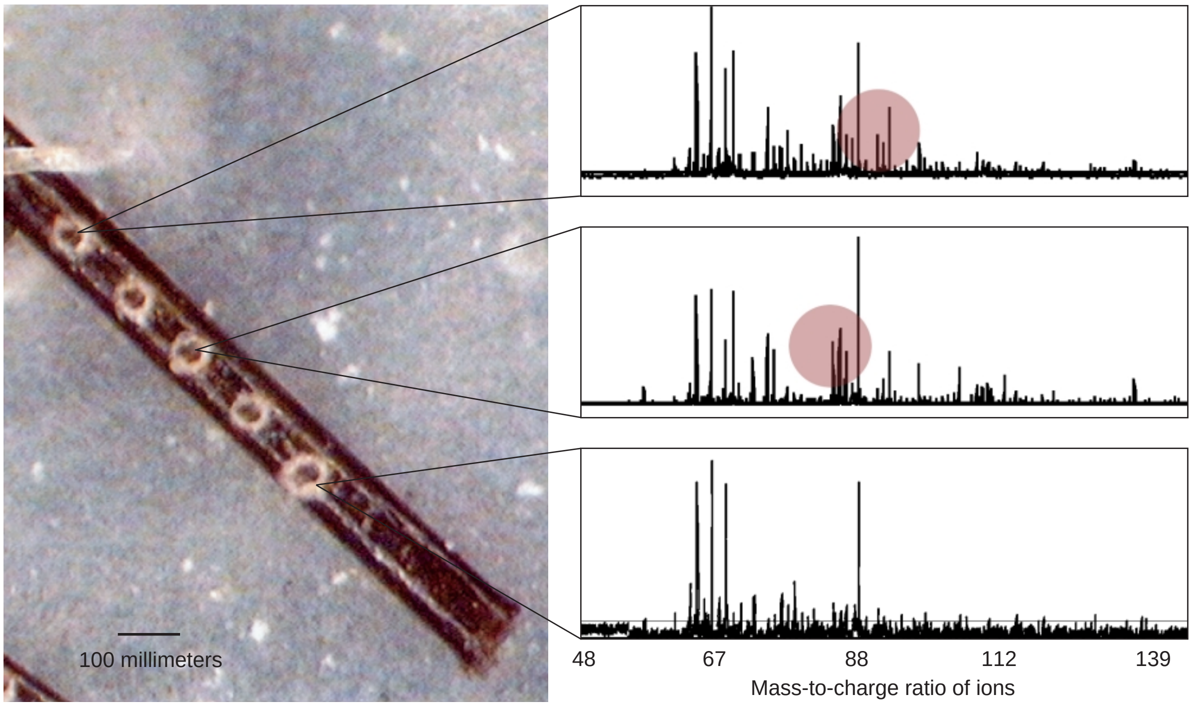
FSC came to the aid of Kings County, California, authorities who were stymied by an execution-style triple homicide. The evidence included a variety of bullet fragments but no weapons. Investigators found corroded, expended casings scattered around the grounds where the suspects lived. FSC personnel led by Rick Randich chemically treated the casings to remove corrosion and then used optical and scanning electron microscopes to match the crime-scene evidence with residence specimens. The staff published its restoration methods as an aid to other agencies.

The center analyzed debris from an explosion that killed a scientist during a 1992 cold fusion experiment at SRI International in Palo Alto, California. In testing the explosion debris, FSC chemists discovered a trace amount of oil in the interior of the SRI electrochemical cell. They determined that a likely source of this oil was lubricating fluid that remained from machining the metal cell components. They concluded that the high-pressure oxygen atmosphere of the electrochemical cell possibly created the potential for an explosive reaction with the oil.

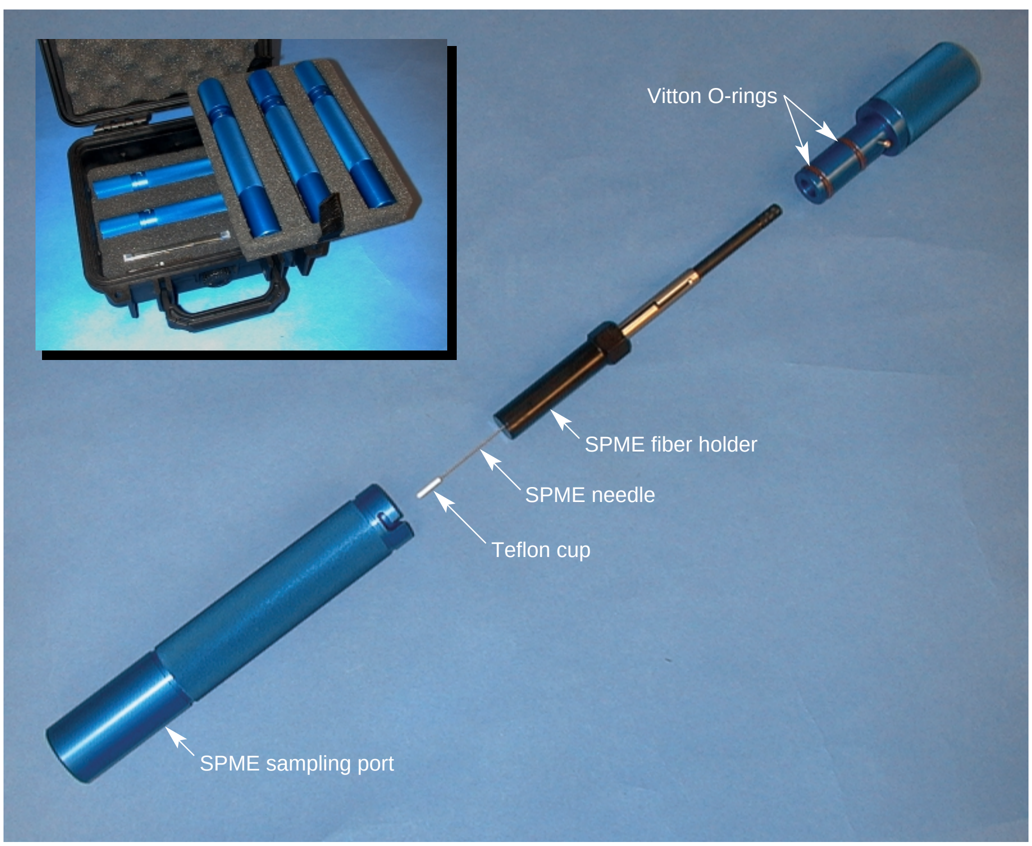
Many FSC investigations involve identifying unknown substances. One specimen brought to the center was a suspicious green liquid uncovered by the Federal Bureau of Investigation (FBI) during a search of a stolen cache of weapons. The container of the liquid was labeled "poison" and gave a dilution formula for use. FSC chemists analyzed the solution for chemical warfare agents but finally identified it as a concentrated cleaning agent.

Another extraordinary analysis centered on a shipment of white crystals in ampoules from China that was thought to be heroin. The powder was interdicted by the U.S. Customs Service and subsequently investigated by the FBI. FSC analyses identified the material as tetrodotoxin, a deadly marine neurotoxin derived from puffer fish. "The definite identification of tetrodotoxin was a real success story for the center," says Andresen.

In the past several months, FSC has been helping authorities to identify samples of substances suspected of being anthrax. Several of the specimens brought to the center by law enforcement officials were from the local community, while others were from locations at the Laboratory. None was found to be real anthrax; instead, the powders were determined to be food materials, dust, dirt, cell culture medium, and powdered paper.



The imaging laser-ablation mass spectrometer allows an investigator to "walk down" a hair shaft by drilling consecutive holes with the laser on the same hair and analyzing each to obtain a volatile sample for a history of activities such as drug use or exposure to chemical weapon compounds.



The Forensic Science Center's solid-phase microextraction (SPME) collection kits use optical fibers as "chemical dipsticks" and (inset) rugged aluminum transport tubes for safe and efficient sampling. The technique has revolutionized collecting evidence in the field.

Chambers uses SPME's coated fibers to collect volatile and semivolatile molecules that are formed or outgassed from the nuclear and thermal breakdown of organic polymers and high explosives. Signs of outgassing can indicate problems such as corroded metal parts that need to be replaced. By monitoring for the presence of these chemical vapors, scientists are alerted to problems that may be developing inside the weapon.

The center has provided the FBI and other agencies with SPME field kits for the safe and rapid collection of chemical warfare agents. The kits are equally well suited for drug detection and arson investigations. FSC has also developed a new SPME transport tube that is smaller and lighter so that it can fit inside a shirt pocket. Both versions are being licensed to industry for sale to government agencies.

This solid-phase microextraction device is used to collect molecules that are formed or outgassed from the nuclear and thermal breakdown of organic polymers and high explosives contained in nuclear warheads. Signs of outgassing can indicate problems with parts that need to be replaced.

Always On Call

Although the Forensic Science Center was highlighted in a Tom Clancy novel, it is not fiction. It is a rich resource for the national security and intelligence communities and has proved itself a valuable ally to federal and state agencies alike. Just as they have for the past 10 years, FSC personnel will be on call for the next case and the next sample.

—Arnie Heller

Key Words: anthrax, Chemical and Biological National Security Program, Forensic Science Center (FSC), gas chromatograph–mass spectrometer (GC–MS), laser-ablation mass spectroscopy, Organization for the Prohibition of Chemical Weapons (OPCW), solid-phase microextraction (SPME), stockpile stewardship, thin-layer chromatography (TLC).

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