Primitive creatures aid healthy space travel

Astronauts onboard the International Space Station will soon be able to test a high-tech medical device that uses primitive enzymes from horseshoe crabs to diagnose human illness.

Symptoms developed while in space, such as a sore throat and fever, require a diagnosis and possibly a dose of antibiotics. If so, which kind? A new biological laboratory on a chip being developed at NASA Marshall, in partnership with outside researchers, could provide the answer in as little as five minutes. The partnership was a four-year-long collaboration among NASA, the Carnegie Institution of Washington, D.C., the NASA Astrobiology Institute, and the Charles River Endosafe Labs, in Charleston, S.C.

The minilab goes by the acronym LOCAD-PTS, which stands for Lab-On-a-Chip Application Development–Portable Test System. The latest version of this lab is a handheld device slated for testing on the space station. It was delivered via the shuttle Discovery last December.

LOCAD-PTS can provide a diagnosis quickly in an environment where time matters.

Timely diagnosis

On Earth, the most reliable method of diagnosing illness is to take a sample of bodily fluid (throat swab, or blood or urine sample) and culture it in a laboratory on Petri dishes with different growth media. Culturing decisively reveals whether an infection is viral (which will not respond to antibiotics) or bacterial or fungal (which will). Culturing also pinpoints the species and thus the most effective kind of antibiotic.

"So-called gram-negative bacteria, such as E. coli and salmonella, respond to different antibiotics than gram-positive bacteria, such as staphylococcus," explains Ginger N. Flores, LOCAD project manager at Marshall.

However, cultures can be impractical, chiefly because growing them can take two or three days. In that time a person might become very ill. LOCAD-PTS, on the other hand, is fast: "It gives results in 5 to 15 minutes," says Norman Wainwright, the project's principal investigator and the director of research and development at Charles River Labs. "And it is very sensitive. It can detect just a single bacterium."

Crabby enzymes

The high-tech device relies on four enzymes extracted from the blood cells of one of Earth's most ancient living creatures—the horseshoe crab. "The horseshoe crab, a species that has survived some 300 million years, has a very primitive but sensitive immune system," Wainwright explains. A single bacterium can be enough to trigger enzymes in the crab's immune system, which clot the blood to seal off a wound.

The enzymes' extraordinary sensitivity and rapid response make them widely useful in medical research to test the effectiveness of drugs and devices. Withdrawing a bit of blood annually from horseshoe crabs, which are then returned to the sea, does not injure the creatures. So far, there is no acceptable synthetic substitute.

It is these horseshoe crab enzymes that allow LOCAD-PTS to be so small, sensitive, and fast. First, a tiny amount of enzyme is inserted into tube-like channels and dried. Introducing any liquid sample to be tested into the channels will cause the enzymes to be rehydrated. If the sample includes bacteria, their toxins trigger the enzymes, which change the liquid's color. The degree of color change depends on the number of germs.

Out-of-this-world testing

The experiment package will compare LOCAD-PTS to tried-and-true Petri-dish methods. Once a week for six weeks, astronauts on the ISS will press a sticky, flexible patch onto an exposed surface, and then swab a short distance all around the patch. Any bacteria adhering to the swab will be introduced into LOCAD-PTS; any bacteria adhering to the sticky patch will be cultured. A few days later, bacterial colonies will be counted in the traditional manner.

This procedure takes advantage of the fact that humans are not the only travelers in space. The surfaces of the space station, just like the walls, floors, and kitchen counters here on Earth, are rich in bacteria.

Wainwright and Flores expect that LOCAD-PTS will prove to work quickly. However, a big unknown is how well it will compare in analyzing the type of bacteria, and how well its color changes can be calibrated to the number of bacterial colonies that grow. "We also want to see how easy and practical the device is for astronauts to manipulate in microgravity," Flores explains.

LOCAD-PTS project scientist Jake Maule from Carnegie Institution and Wainwright tested the LOCAD-PTS in zero-g during parabolic flights of NASA's DC-9 aircraft in April 2006. All aspects of LOCAD-PTS procedures, especially the fluid handling tests, were thoroughly evaluated in a zero-g environment before flight to the ISS.

The initial LOCAD-PTS will test only for gram-negative bacteria. Scientists plan to use the device later to test for gram-positive bacteria, yeast, mold, and certain chemicals.

A microfluidic chip is being developed as the next generation of LOCAD-

PTS cartridge. It is miniaturized even further, to allow many more tests to be completed simultaneously. This increased capability will save the crew time and allow more sophisticated types of analyses to be performed, ranging from medical tests to monitoring potentially hazardous chemicals on board the ISS.

Eventually, if all goes as planned, one sample from the throat of an ill-feeling astronaut could be sent flowing through parallel channels on a future generation of LOCAD-PTS and, within minutes, a diagnosis could be made.

Not just protecting, but finding life

Beyond advising future astronauts regarding types of illnesses, the potential role for the lab on a chip is to help protect space explorers and detect life forms on Mars.

Dime-size lab-on-a-chip technology allows chemical and biological processes previously conducted on large pieces of laboratory equipment to be performed on a small glass plate with fluid channels, known to scientists as microfluidic capillaries. "We are studying how lab-on-a-chip technology can be used for new tools to detect bacteria and life forms on Earth and other planets and for protecting astronauts by monitoring crew health and detecting microbes and contaminants in spacecraft," explains Helen Cole, project manager for the LOCAD program.

The chips are made with the same microfabrication technique used to print circuits on computer chips. Chemicals and fluid samples can be mixed, diluted, separated, and controlled using channels or electrical circuits embedded in the chip. On Earth, some basic lab-on-a-chip technology approaches are being used for commercial medical diagnostic applications, such as an in-office test for strep throat or modern in-home pregnancy tests. These applications conduct a test and yield results in a short time, with a handheld portable device containing a simple chip design.

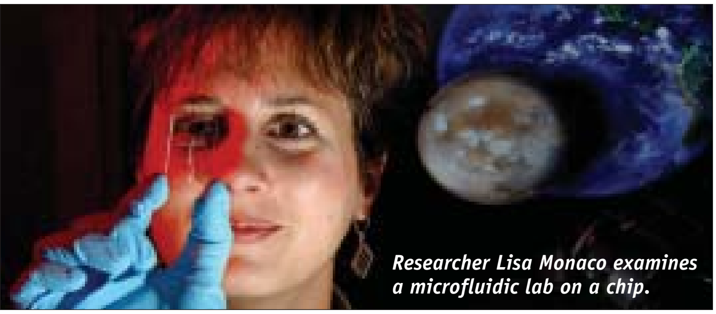
"NASA requires complex lab-on-a-chip technology so scientists can conduct multiple chemical and biological assays or can perform many processes on a single chip," says Cole. "Current commercial devices are not designed to work in space, so we are developing a set of unique chips along with a corresponding miniaturized controller and analysis unit."

NASA researchers are developing complex, portable micro array diagnostic chips to test for all the genes and DNA responsible for determining the traits of a particular organism, detect specific types of organisms, or use biosensor-like probes such as antibodies to detect molecules of interest. By applying this technology in laboratories and in the field where organisms live in extreme environments on Earth, astrobiologists can compare Earth-life with that which may be found on other planets.

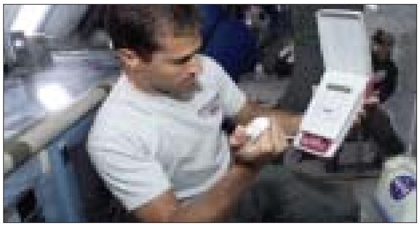
"The micro array chip system developed to go to Mars will be lightweight, portable, and capable of detecting organic molecules," says Lisa Monaco, the project scientist for the LOCAD program.

Since the chips are small, a large number of them can be carried on a Mars rover to search for life or carried on long-duration human exploration missions for monitoring microbes inside lunar or Martian habitats.

"We need customized micro array chips to find and characterize life at remote places on Earth, Mars, and other places in the solar system," says Andrew Steele, a scientist at the Carnegie Institution. Steele, the principal investigator for the Modular Assays for Solar System Exploration (MASSE) project, is working with Marshall scientists and engineers to develop the technology and instruments needed to analyze samples quickly and produce images of samples.



Researcher Lisa Monaco examines a microfluidic lab on a chip.



Researcher Jake Maule tests LOCAD-PTS.

"When astrobiologists study life in extreme environments—whether it lives deep in the ocean, in Antarctica, or on Mars—they need a handheld device or something that can fit on a small robot," Steele explains. "We also need to be able to analyze the tests as quickly as possible, within periods from 1 to 24 hours. Marshall is one of just a few places in the world developing these specific technologies for space and exploration applications and has unique experience in miniaturizing these instruments and designing them for the harsh space environment."

Chips aplenty

The Marshall team is collaborating with scientists at other NASA centers and at universities to design chips for many applications, such as studying how fluidic systems work in spacecraft and identifying microbes in self-contained life support systems.

To make customized chips for these various applications, NASA has an agreement with the U.S. Army's Microdevices and Microfabrication Laboratory at Redstone Arsenal in Huntsville. The lab-on-a-chip research is funded by NASA's Biological and Physical Research Enterprise through Marshall's Microgravity Science and Applications Division.

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