PCR heads into the field

Vivien Marx

Analyzing samples with PCR is routine in the lab. New approaches let researchers do this assay wherever they need.

Biomedical researchers working in the field spend much of their time outdoors, and they need their instruments with them. Now there are new ways for them to apply the polymerase chain reaction (PCR) even when there are no classic lab benches or electrical outlets nearby. They need these mobile PCR approaches because samples can degrade on the trip from a remote location back to the lab. And in many settings, scientists and their collaborators want to act on results quickly.

The new, portable devices and approaches let scientists look for specific genetic material in their samples as they hunt for pathogens affecting dynamic ecosystems, screen migrating people for infectious diseases or analyze rapidly changing water conditions. Some devices are prototypes; others are already being sold. Some designers use standard PCR instruments, others have developed new miniaturized hardware and still others avoid using instruments entirely.

Institut Pasteur in Cambodia, Ménard lab

Scientists at Institut Pasteur in Cambodia designed a mobile lab that can be towed to remote villages to quickly detect malaria in many people.

PCR in rural Cambodia

In 2013, according to the World Health Organization, there were about 198 million cases of malaria and around 580,000 related deaths around the world. Treatment and prevention has led to a drop in malaria deaths: there were 4.3 million fewer deaths between 2001 and 2013 compared to what might have happened if the incidence had stayed at 2000 levels. Five parasite species are capable of infecting people with malaria, of which *Plasmodium falciparum* is the most deadly.

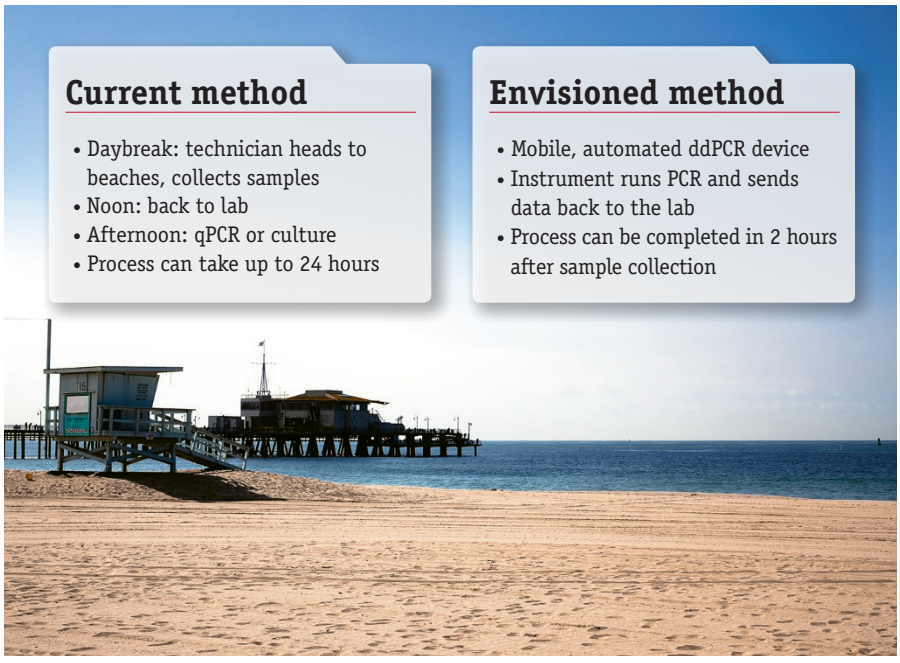
But malaria remains difficult to eradicate, for many reasons. Even when transmission of the parasite *Plasmodium* via its mosquito vector is much lowered, for example in Cambodia, a parasite reservoir remains. This reservoir is in infected but asymptomatic people with low levels of parasites in their blood. Complete malaria eradication, says Didier Ménard, a molecular epidemiologist at Institut Pasteur in Cambodia, means finding these people, especially migrants. "As they move we need to test and treat in a delay as minimum as possible," says Ménard.

Detecting low parasite densities—also called parasitemia—which involves microscopy-based examination of blood and PCR-based assays, takes 5–7 days, a time-frame that includes shipment of samples to a central lab. Ménard and his colleagues found a way to do assays in the field and get results within 48 hours for their trip through the impoverished villages in the Ratanakiri region in eastern Cambodia, where malaria is a contributor to the high infant mortality rate. Electricity there is either irregular or completely absent[1].

In collaboration with a company called FEMIL, the researchers designed and built a mobile lab, a kind of shipping container with a floor plan of around 15 square meters and that can be towed by a truck. The truck accommodates an electrical generator, and the mobile lab has room for an office, a parasite culture room with freezers, an incubator and a microscope. There is a separate room devoted exclusively to PCR assays: it holds two real-time PCR instruments as well as two other PCR instruments and a hood with a UV spectrophotometer[1].

The researchers developed assays to obtain genus-specific results for four *Plasmodium* species based on the *Plasmodium* cytochrome *b*–encoding gene. They first use PCR to find samples positive for malaria and then do a second round of PCR with species-specific primers.

Ménard and his team traveled to nearly 100 eastern Cambodian communities, testing between 200 and 300 samples and 40 quality-control samples a day in their mobile lab. From a finger prick of blood, says Ménard, the PCR assays detected levels as low as one parasite per 5 microliters (μl)



SCCWRP (source) and Erin Dewalt/Nature Publishing Group (graphic) and Giorgio Fochesato/iStock/Thinkstock

Researchers at the Southern California Coastal Water Research Project (SCCWRP) and colleagues are working on ways to detect microbial contaminants in water more rapidly.

of blood. In his view, their work shows the feasibility of detecting low infection levels on a massive and mobile scale, which should help to eradicate malaria in certain regions. Administering drugs to many people independent of infection status risks increasing drug resistance, says Ménard. This mobile strategy finds only the people who need to be treated and therefore saves time and money, he says.

Ocean PCR

When bacterial levels are high in ocean water close to beaches, community officials post warning signs for swimmers. But the testing process takes too long.

Scientists at the Southern California Coastal Water Research Project (SCCWRP), a public agency that develops and evaluates technologies for ocean monitoring, is exploring how to use droplet digital PCR (ddPCR) to detect microbial contaminants in water more rapidly. Together with the Monterey Bay Aquarium Research Institute and Arizona State University, they are developing new mobile ddPCR technology.

They want to equip the new device with the ability to send results to the lab. The team expects to have a prototype instrument capable of this telemetry later this year, says John Griffith, a microbiologist with SCCWRP. Among the validation tasks he plans is to test and compare the output to results from the Bio-Rad benchtop ddPCR instrument.

The mobile automated ddPCR instrument will deliver speedier and more accurate microbial water-quality measurements so that public health warnings can be relayed to bathers within two hours of sample collection. Right now it takes much longer: a technician travels to the beach at daybreak, collects water samples, returns to the lab by noon, and then analyzes the sample for fecal indicator bacteria (FIB). These indicators are typically *Escherichia coli* or *Enterococcus*, which serve as surrogates for pathogens. The analysis may be done either by culturing the FIB or, more rarely, by quantitative PCR (qPCR).

Analysis of samples by culturing FIB is slow. The cultures must typically grow for 18–24 hours before they produce an actionable result. But during the time needed to run the assay, swimmers might be exposing themselves to potential pathogens. Also, ocean water quality changes quickly.

qPCR can accelerate testing and can also get results in earlier in the day. "If everything went right, then the qPCR results were ready by around 11:00," says Griffith. But producing a result this way is still too expensive. Someone has to collect samples and bring them back to the lab. It would be cheaper, says Griffith, if a technician or a lifeguard could load and analyze samples in a mobile ddPCR device and the data were transmitted back to the lab or to a health department.

It is too difficult to test for all possible pathogens, so FIB serve as proxies, says Griffith. High levels of human or cattle waste can pose an infection risk to humans, but FIB can also be from bird feces or decaying kelp, which pose a much lower risk. This diversity is why the mobile ddPCR instrument is so helpful, says Griffith, because it can deliver quantitative measurements, can tell officials whether the bacterial contamination source is likely human, and can help to pinpoint the location of the source.

The mobile ddPCR instrument will be about the size of a small suitcase that can be mounted onto a beach water sampler's vehicle or operated by a nonscientist such as a lifeguard. Once samples are loaded, DNA can be extracted, followed by PCR and analysis. The data will be sent back to the lab or health department. Given the many types of primer or probe combinations, it will be possible to track specific fecal contamination, says Griffith. And the contamination signal can be followed in real time.

The instrument could also help detect disease-causing pathogens in marine and brackish waters where shellfish are harvested. Organisms such as *Vibrio parahaemolyticus* and *Vibrio vulnificus* lead to seafood poisoning when people eat an infective dose of shellfish containing a concentrated amount of these bacteria. "There is also growing concern that pathogenic *Vibrio* species may proliferate in California waters as ocean temperatures continue to rise due to climate change," says Griffith.

As the device evolves, the team wants to make it available to all, by lending it to collaborators or helping them build or acquire some of their own, says Christopher Scholin, CEO of the Monterey Bay Aquarium Research Institute. Eventually, and if the instrument proves successful, the hope is to find a commercial partner to provide and support the system.

Remote PCR is useful for many aspects of ocean and environmental science, says Scholin, and is not just for chasing bugs that pose health hazards to humans and wildlife. The device might even one day be integrated into an autonomous underwater vehicle equipped with many sensors, though engineers still have to make the devices sturdy enough for long-term use on the high seas, says Griffith.

Creating a network of such sensing devices, which do not require a person to always be present, will lend researchers the ability to "track and trend" indicators of

various kinds, such as of the state of a particular region, Scholin says.

Life in the ocean is based on ephemeral events, changing constantly over time and horizontal and vertical space. Sampling those events to gain an understanding of what makes ecosystems tick is challenging when you only can spend a limited amount of time at sea, he says. "Even if you pack up your lab and set it up on a ship, for example, you're still going to miss most of what's going on hour by hour, day by day, and it gets harder the more you travel to remote and extreme environments," says Scholin. "We can't beat the sampling problem by throwing more people and ship time at it; it's too expensive and unsustainable." That is why, he says, it is valuable, whenever possible, to move the laboratory from the ship into the environment itself and send data, not samples, back to centralized facilities for interpretation and dissemination.

PCR with new hardware

Faster PCR analyses are also beneficial for forensics applications and investigating suspected acts of bioterrorism.

At the Lawrence Livermore National Laboratory (LLNL) Center for Micro- and Nanotechnology, microfluidics engineer Reginald Beer and his colleagues have developed a PCR thermal cycling method intended for especially fast use in the field.

The team reports amplifying DNA—namely, a synthetic sample of a portion of severe acute respiratory syndrome (SARS)-associated viral DNA and bacterial genomic DNA—in specially devised hardware that can perform near-instantaneous heating and cooling of 5-µl reaction volumes. The technology involves porous material and convective heat transfer of a thermal fluid such that heating and cooling rates of 45 degrees Celsius per second are achieved[2]. The researchers tested a number of polymerase enzymes and found them able to handle this speed, too.

Since their first publication, the team has built a more compact and rugged second prototype, says Elizabeth Wheeler, a chemical engineer and the Bio-Engineering and Detection Group Leader at LLNL, who has worked with Beer. The team is now making the instrument able to run on battery-generated power. They want to make the electronics smaller, too, so that the device is about the size of a shoebox.

This technology can be applied in biodefense, in physicians' offices and for environmental monitoring and forensics applications. "Given the speed of amplification, we are now entering the realm where all those forensic dramas where identifications occur during a commercial break are not so unrealistic," says Wheeler.

Handheld PCR

PCR instruments can be too costly for many field applications, and several groups are tackling this challenge. OpenPCR is one company selling open-source hardware for a low-priced thermocycler that has a 25 cm × 13 cm footprint and a 200-µl sample capacity. The company says it takes five hours to assemble the parts they sell, which include the heat sink, fan, electronics, lid heater, block temperature sensor and power supply box.

A company called Ubiquitome, a spinout of the University of Otago, has developed an in-field quantitative real-time PCR device called Freedom 4. It has a four-well sample strip, a thermal cycler and optics in a package that has a 4 inch × 8 inch (10.2 cm × 20.3 cm) footprint.

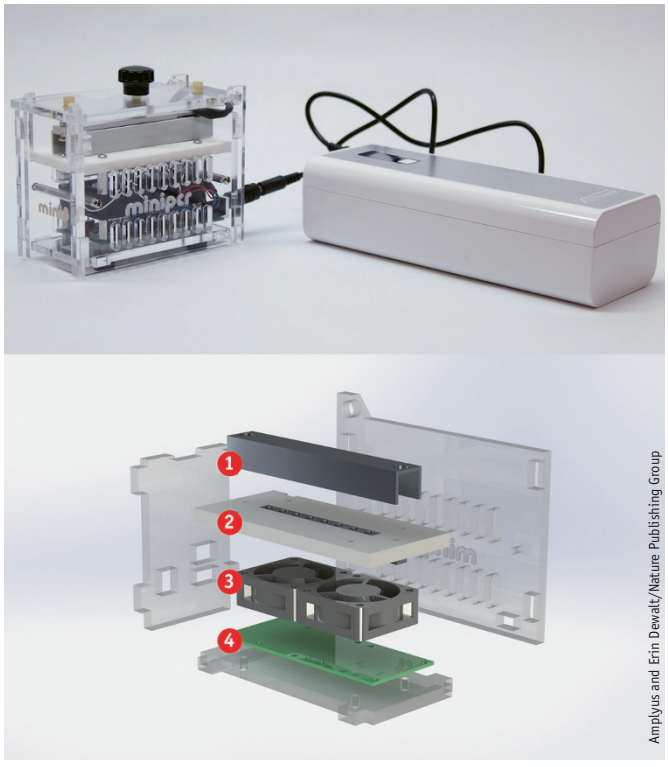
Another mobile PCR development comes from molecular biologist Ezequiel Alvarez Saavedra and neuroscientist Sebastian Kraves, who founded the start-up Amplyus. With financing from family and a Kickstarter campaign, the team has designed and built miniPCR. The mobile PCR instrument has a 2 inch × 5 inch footprint (5.1 cm × 12.7 cm), weighs less than a pound and uses an electrical outlet or batteries that keep the device running for six hours. It uses standard consumables, reagents and experimental protocols, all to permit users to draw on PCR approaches they know well, says Saavedra.

It was demanding to engineer all the components into a small package. "We had to simplify the technology rather than make it more complex," says Saavedra. "Innovation often tends to go in the opposite direction." The device can be operated with a mobile phone or tablet or through a Mac- or Windows-based computer. Feedback has been good thus far, and he and his colleagues hope to continue to evolve the technology.

In 2014, Amplyus sold nearly 500 units to customers ranging from citizen scientists and science teachers to researchers and physicians. One doctor in Sierra Leone uses it in Ebola diagnostics, says Saavedra.

Another miniPCR user is Ilio Durandis, who founded the Haitian Bioscience Initiative to train bioscience lab technicians and scientists. Amplyus gave him a miniPCR and primers, with which he teaches young Haitians how to extract DNA from strawberries and then run gel electrophoresis. The advantage of miniPCR is cost and size, says Durandis. Wherever he has a laptop and a power source, he can start working and see the graph of his PCR analysis on-site.

Durandis also uses miniPCR to monitor water quality. During the 2010 cholera outbreak in Haiti, government officials lacked the technology to detect the *Vibrio cholerae* strain causing the disease. In case of future



Amplyus and Erin Dewalt/Nature Publishing Group

Scientists at the startup Amplyus designed and built miniPCR. The layers show the heating lid (1), heating block for eight DNA samples (2), high-speed cooling fan (3) and open-source electronics (4).

Biomeme and Erin Dewalt/Nature Publishing Group

Biomeme's portable PCR device, currently with researchers for beta testing, docks into the iPhone.

outbreaks, he says, miniPCR will help him and others detect whether water is contaminated.

Biomeme is another company with a moble PCR device. One of its instruments, currently in beta-testing and not yet for sale, is in the mountains of Peru with molecular biologist Tracie Seimon. (Owing to her field research, she could not comment for this story.) Seimon is with the Wildlife Conservation Society and is based at the Bronx Zoo. She uses the device to explore why entire populations of amphibians have been wiped out by a pathogenic chytrid fungus, says Maria Chacon-Heszele, a biochemist at Biomeme. "It's a very nasty pathogen," she says. "It's exciting to work with the Bronx Zoo where there is a specific need for a 'lab-in-a-box.'"

Biomeme's device is a portable, battery-powered real-time PCR machine with thermocycler and fluorometer. It weighs less than a pound, is about the size of a soda can, and docks into an iPhone that runs an app controlling the device's electronics. Marc DeJohn, who cofounded the company, says his tool-building approach is part of the emerging biohacker movement, which explores disruptive ways of developing cheaper tools for biologists. One early Biomeme investor is Jared Tarbell, a software engineer and cofounder of Etsy, a website for handmade goods.

Along with the device, Biomeme offers a sample prep kit for nucleic acid extraction without a centrifuge. The team freeze-dries consumables to increase their shelf-life, avoid the need for refrigeration and cut down on volume. Biomeme wants to work with scientists to create tailored, field-ready, freeze-dried assays with an individual lab's favorite primers and probes.

The iPhone's camera sensor helps with fluorescence detection, and the device uses the iPhone's interface, which is more user-friendly than many scientific instruments, DeJohn says. The first-generation device is physically connected to the iPhone to cater to biodefense beta testers seeking to avoid radio signals, but future units will be detachable.

DeJohn and his team designed the thermocycler to be compact, to offer precise temperature control and to be amenable to mass-fabrication techniques used in electronics. The thermocycling core is the priciest part of a PCR device and usually involves Peltier heat pumps in which arrays of thermocouples move heat from one side to the other. But they are expensive and energy inefficient, says DeJohn, who has worked in instrument development for over a decade.

The Biomeme thermocycler uses a tiny fan for cooling. It heats electrothermally with surface-mount technology, which is common in electronics fabrication. The optical assembly has also been tweaked for cost. After the next round of feedback, the team plans to build around 80 devices, which will go out to a wider circle of beta testers in the biomedical, ecology and biodefense communities.

The device is also being tested in a clinical trial on diagnosing sexually transmitted diseases. "It's a blinded study, so we will not have results until later this year," says Chacon-Heszele.

No-hardware PCR

PCR requires thermocycling, which tends to be powered by electricity. But power can be hard to find with mobile applications and in resource-poor regions.

A group of bioengineers at Rice University avoids hardware by using a lateral flow strip and armpit incubation for isothermal nucleic acid amplification. They use body heat to incubate reactions for recombinase polymerase amplification, for example to amplify DNA from HIV-1 in a controlled reaction[3]. The scientists tested several ways of fastening PCR tubes to the body: taped to the stomach, held in the fist, placed in a pants pocket, and fastened to the armpit with a small holster made of a cloth strip. The armpit turned out to have the steadiest temperature.

Such isothermal nucleic acid amplification techniques use one constant temperature instead of the multiple heating and cooling cycles in PCR. The group decided on recombinase polymerase amplification, an isothermal approach that lets them amplify impure samples, works at around 31 degrees Celsius and can amplify DNA in as little as five minutes.

Separately, in their work on neglected tropical diseases such as river blindness, researchers on the New England Biolabs

Haitian Bioscience Initiative

Ilio Durandis (in orange shirt), founder of the Haitian Bioscience Initiative, trains lab technicians and scientists using miniPCR and primers.

(NEB) campus (affiliated with the company) also developed an example of a device-free amplification approach[4]. Nathan Tanner, an applied molecular biologist at NEB who develops enzymes and biomedical technologies and who led the effort, paired isothermal DNA polymerases developed at NEB with a visual colorimetric readout of the amplification reaction.

In DNA replication, polymerases produce a proton as each deoxynucleotide is incorporated into the new strand, says Tanner. The team enabled amplification reactions with buffer and pH-tolerant enzymes. There was a 2–3 unit drop in pH just due to the output of the DNA polymerase. "By coupling this pH change to classical pH indicators, we get a clear, robust visual detection of amplification," he says.

The method can detect as few as ten copies of DNA in under 30 minutes. "That's the kind of sensitivity and speed that real field assays need to find widespread adoption," says Tanner. Often, disease-causing targets are present with only a few copies. NEB is also testing the technique with the Water Research Institute in Ghana.

The NEB team chose loop-mediated isothermal amplification (LAMP). They tested other amplification strategies such as strand displacement amplification, but their proof-of-concept testing was mainly with LAMP. "LAMP is popular in the literature, and we produce a number of enzymes that work well in LAMP," says Tanner. "It seemed like a good fit for this study."

In neglected tropical diseases, it is best to test an infected person and be able to treat immediately, especially given that many peoples are seminomadic, says Clotilde Carlow, an NEB parasitologist who is part of the project. But tools for diagnosis and insect surveillance either are not sensitive or not specific enough or are too costly.

Field diagnostics must be simple and robust for hot, humid regions, and they need to be accurate and affordable. Conventional PCR machines do not travel well, but Carlow acknowledges the emergence of smaller, handheld devices. "Another issue with PCR is the requirement for post-reaction manipulation, and additional equipment to detect the amplification product," she says.

Tanner says that the initiatives to create portable and miniaturized PCR machines such as those from Biomeme and Ubiquitome are promising ways to advance both field and point-of-care testing. The NEB team has not been focusing on instrumentation but rather on the enzymes and the biochemical machinery that enables these reactions. "We're using a direct readout of the enzymatic reaction without any hardware, making it much easier to carry hundreds of tests in a backpack," he says. LAMP detection configured in this way, he says, needs only a sample, warm water, and eyes.

To accomplish their work, field scientists have to pack up their lab to come along. With approaches such as miniaturized instruments and device-free methods, nucleic acid amplification and analysis have become possible in the field. PCR, a standard assay, is going places.

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Vivien Marx is technology editor for *Nature* and *Nature Methods* (v.marx@us.nature.com).