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The Sooner, The Better

New approaches to diagnosing bacterial infections may one day allow the identification of pathogens and their antibiotic susceptibility in a matter of hours or minutes.

By Nicholette Zeliadt | July 1, 2014

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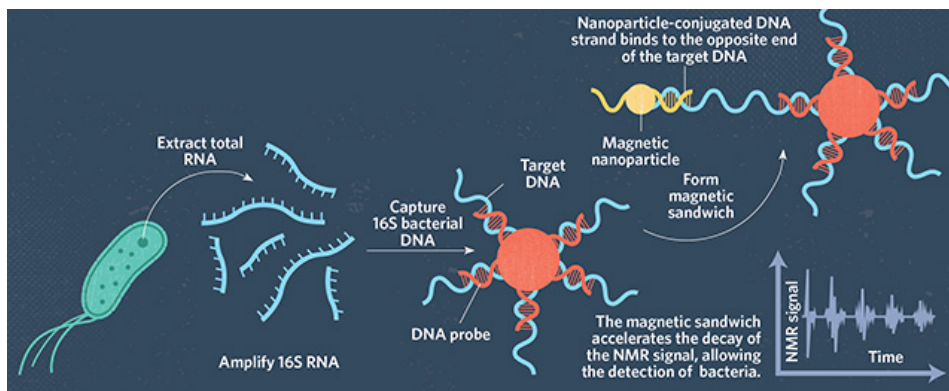
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MAGNETIC DETECTION: To identify bacteria by NMR, researchers first extract total RNA from bacteria and use PCR to amplify the 16S RNA as 16S DNA, one end of which can be captured by microbeads (1–3 μm in diameter) coated with a covalently bound DNA probe. DNA conjugated to magnetic nanoparticles (20–30 nm) binds to the opposite end of the target DNA, forming a magnetic DNA sandwich that can be detected by nuclear magnetic resonance (NMR).

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When someone shows up at the doctor's office with signs of an infection, chances are good that the physician will diagnose the ailment using methods that have changed little during the past century. After inquiring about symptoms, the doctor might collect a sample of blood, urine, or some other bodily fluid and send it to a clinical lab where it will be cultured and examined under a microscope. If bacteria are present, the lab might conduct additional culture-based tests to determine the species and its susceptibility to various antibiotics.

Depending on the microbe's propensity to grow in culture and the amount of it present in the sample, it can take a day or longer for the lab to identify the pathogen, and an additional one to two days to get the antibiotic susceptibility results. But in many cases it can be impractical or risky for a clinician to wait for those results before initiating treatment. Therefore, he or she will make an educated guess as to the cause of the infection and might send the patient home with a prescription for a broad-spectrum antibiotic capable of ridding the body of a number of bacterial pathogens.

This practice means that doctors sometimes prescribe antibiotics when they are not necessary, potentially leading to harmful side effects and contributing to the emergence of antibiotic resistance. In other instances, when a bacterial infection is resistant to the empiric antibiotic therapy, delays in treatment with an appropriate antibiotic may increase the risk that the infection will worsen or spread to others. "If there were rapid diagnostics available, we could provide more-targeted treatments sooner, rather than later," says [Joseph Liao](#), associate professor of urology at Stanford University's medical school and the chief of urology at the VA Palo Alto Health Care System in California.

To speed the diagnosis of infections, Liao and others are developing simple and inexpensive tests based on biosensors that convert the molecular recognition of a target molecule into signals that can be measured optically, electrically, mechanically, or magnetically. Well-known examples of commonly used biosensor-based assays include home pregnancy tests and handheld glucose monitors. Biosensor technologies for infectious diseases still need to clear significant regulatory hurdles before they can be used clinically, but they already show promise as portable and easy-to-use tools to rapidly identify a variety of pathogenic bacteria and to inform effective treatments. Here, *The Scientist* looks at three such technologies under development.

Magnetic DNA Sandwich

Researchers: [Hakho Lee](#), assistant professor, and [Ralph Weissleder](#), director, Center for Systems Biology, Massachusetts General Hospital, Harvard Medical School

Technology: Using a miniaturized nuclear magnetic resonance (NMR) device created by Weissleder's lab, Lee and Weissleder's team developed an assay that can determine whether or not a sample contains

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virtually any species of bacteria or specifically identify 13 different bacterial pathogens in about two hours' time ([Nat Nanotechnol](#), 8:369-75, 2013). The researchers first isolate RNA from bacteria and use PCR to amplify a 50-70-nucleotide region of the 16S ribosomal RNA—either a region that is highly conserved among most bacterial species (for universal detection of bacteria), or a sequence that is unique to a particular pathogen of interest (for species-specific detection). Then they mix a sample of the PCR reaction with microbeads covalently coated with DNA probes 18-22 nucleotides in length that are designed to bind to one end of the PCR product. After washing away unbound DNA and beads, they add magnetic nanoparticles conjugated with DNA strands that bind to the opposite end of the PCR amplicon, forming a so-called magnetic sandwich complex with the bacteria-derived DNA in the center. "Eventually what we have are microbeads coated with these magnetic nanoparticles," Lee says.

After further washing to rid the beads of unbound nanoparticles, the researchers place the sample inside their miniaturized NMR device. The NMR signal in samples that contain magnetic nanoparticles decays faster than in those that do not, Lee says, thus enabling detection of samples that contain bacteria.

Application: The assay was able to detect as few as one or two bacteria added to a 10-mL sample of whole blood and also provided an estimate of the number of bacteria present in a sample. It also accurately diagnosed a variety of patient specimens—including lung fluid, pelvic abscess contents, urine, and bile—as either positive or negative for bacteria. In samples positive for bacteria, the assay correctly identified all of the bacterial species that could be detected by standard culture, as well as two additional species not detected by culture.

Pros:

- The assay can be performed using as little as 10 μ L of a patient sample.
- The detection step has very low background, because the samples have no inherent magnetic signal.
- The miniature NMR device is small, portable, and inexpensive. Lee says the device itself costs roughly \$500 to build, and estimates that the cost of the magnetic nanoparticles and other reagents is about \$10 per assay.

Cons:

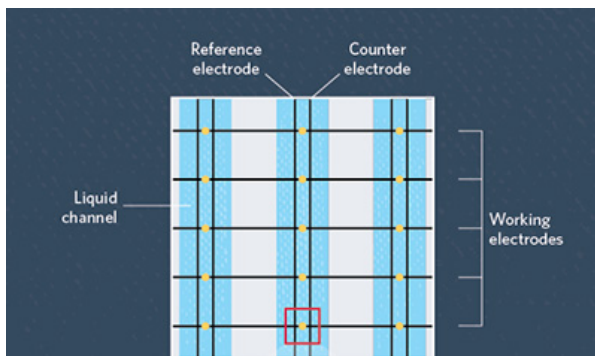
- The assay involves multiple, independent steps that can increase the risk of contamination and inaccurate results.
- No multiplexing. The assay must be repeated for each bacterial species of interest.

Other applications: Using a similar strategy, the group developed an assay for the detection of PCR-amplified DNA from *Mycobacterium tuberculosis*. The assay is performed on a microfluidic chip that handles the PCR, bead capture and labeling, and NMR detection steps ([Nat Commun](#), 4:1752, 2013). In addition, the team has varied the molecules used for capturing and labeling to detect other molecules of interest besides bacterial nucleic acids. For instance, Lee and Weissleder detected cancer cells in patient biopsies using magnetic nanoparticles that glom onto antibodies bound to particular cancer-related proteins present on the cell surface ([Sci Transl Med](#), 3:71ra16, 2011).

Electric Nucleic Acid Test

Researcher: [Shana Kelley](#), professor of chemistry, pharmaceutical sciences, and biochemistry, University of Toronto, Ontario, Canada

Technology: Kelley's group developed an electronic chip that can rapidly detect DNA from 20 different bacterial species that frequently cause urinary tract infections and 10 different genes that confer antibiotic resistance. The chip features a pair of counter and reference electrodes running along the inner surface of each of five liquid channels. Each channel is further subdivided into five separate wells containing an electrocatalytic solution, and an array of 20 so-called working electrodes runs perpendicular to and underneath the wells. Bushy, gold microelectrodes sprout from the working electrodes and into each well, but remain electrically isolated from the counter and reference electrode pair.



CLOSING THE CIRCUIT: In the electronic chip (top), a pair of reference and counter electrodes runs along the bottom of each liquid channel, and working electrodes are situated perpendicular to and underneath the channels; gold microelectrodes (yellow) rise up from the working electrodes and into the channels. Nucleic acid probes (orange, shown in a cross-sectional view of a single microelectrode, middle) immobilized on the microelectrode capture the negatively charged target DNA (navy), attracting cations and triggering redox reactions that generate an electrical current.

See full infographic: [JPG](#) | [PDF](#)
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Each well's microelectrodes are coated with a nucleic acid-based probe designed to bind only to nucleic acid from a specific target pathogen or antibiotic-resistance gene. When a probe binds its target DNA sequence, cations in the electrocatalytic solution are attracted to the negatively charged phosphate backbone of the nucleic acid. The ions in the solution then undergo a series of oxidation and reduction reactions, generating a current that flows between the working electrode and the pair of counter and reference electrodes in the well. The resulting electrical signal in each well can be measured using a simple device called a potentiostat.

Application: The chip was able to detect DNA from bacterial lysates containing as little as one bacterial

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cell per μL . What's more, the electrical signal could be detected after only a two-minute hybridization between the sample and the probes ([Nat Commun](#), 4:2001, 2013).

Pros:

- Multiplexing. Depending on the probes present on the chip, the method can screen a sample for a variety of pathogens simultaneously, or a combination of pathogens and antibiotic-resistance genes.
- Kelley says the reagents needed for the assay are inexpensive, and the chip itself costs only a few dollars to make.
- Very fast.

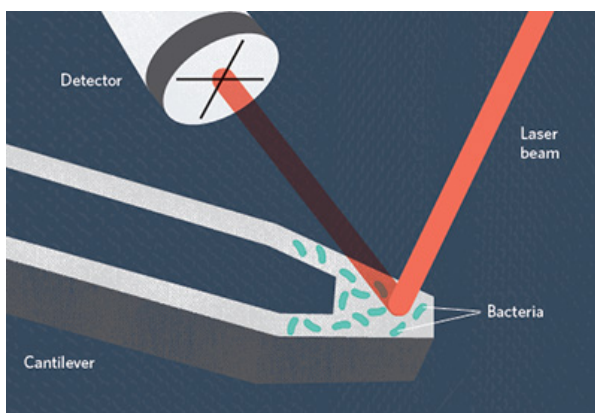
Cons:

- Not yet demonstrated to work with clinical samples
- The detection limit of one cell per μL is not likely to be sensitive enough to detect bacteria in some types of patient samples, such as blood.

Vibe Monitors

Researcher: [Giovanni Longo](#),
École Polytechnique Fédérale de
Lausanne (EPFL), Switzerland

Technology: Using a tiny diving board–like device called a cantilever that's traditionally used in atomic force microscopy (AFM), Longo's group devised a way to rapidly determine bacterial resistance to antibiotics. The researchers coat a chemically treated cantilever with living bacteria and immobilize one end of it inside a chamber filled with bacterial growth medium. The bacteria cause the free end of the cantilever to vibrate at a certain frequency as they move and carry out ordinary metabolic functions. Those nanoscale vibrations are monitored using a laser beam that reflects off the surface of the cantilever and into a detector.



MONITORING VIBRATIONS: A laser beam deflected off the surface of a cantilever can detect the nanoscale movements caused by viable bacteria.

See full infographic: [JPG](#) | [PDF](#)
THE SCIENTIST STAFF; COURTESY OF GIOVANNI LONGO

Upon exposure to an antibiotic, the fluctuations caused by antibiotic-susceptible bacteria grind to a halt within a matter of minutes, and do not recover in the presence of fresh growth medium without the drug. By contrast, the fluctuations caused by bacteria resistant to the antibiotic initially stall in the presence of the drug, but gradually resume as the bugs ramp up the molecular pathways they need to survive the treatment.

Application: Longo's team demonstrated that lab-grown cultures of antibiotic-sensitive *Escherichia coli* and *Staphylococcus aureus*—two of the most common human pathogens that are rapidly developing resistance to many available antibiotics—cease to produce fluctuations of the cantilever within five minutes or less after antibiotic treatment. By contrast, fluctuations caused by antibiotic-resistant strains of the same microbes resumed within 15 minutes of antibiotic exposure ([Nat Nanotechnol](#), 8:522-26, 2013). Although the tests were performed on a traditional atomic force microscope, which can cost hundreds of thousands of dollars, Longo says his team has developed simplified prototype versions of the instrument for \$5,000–\$10,000 that are designed specifically for antibiotic susceptibility testing and that don't require expertise in AFM to operate.

Pros:

- Fast. The team obtained their results for *E. coli* and *S. aureus* in about 30 minutes each. Longo suggests the method may be particularly useful for assessing the antibiotic susceptibility of slow-growing organisms such as *M. tuberculosis*, which does not grow rapidly in culture. Traditional culture-based tests of antibiotic susceptibility for *M. tuberculosis* can require weeks, but using this method, Longo says his team has been able to cut that time down to under four hours.
- AFM cantilevers are fairly inexpensive, selling for around \$5–\$10 each.

Cons:

- Longo's team has not yet tested the technique with clinical samples.
- No multiplexing. Can only analyze one sample of bacteria at a time, though Longo says his group is working on a multicantilever system.
- Cannot yet characterize a mixture that contains more than one bacterial species. "If an antibiotic affects one species but not the other, you wouldn't know what's going on," Longo says, because the drop in movement of the susceptible species would be masked by the fluctuations of the resistant species.

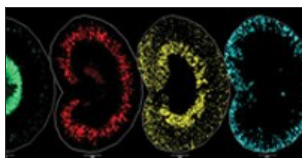
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