Next-Generation Sequencing opening doors in diagnosing perplexing disorders

We've all seen episodes of House and watched the ornery doctor fill his whiteboard with symptoms, risk factors and diseases that might match them. Then comes the battery of tests – no doubt inconclusive – followed by another trip to the whiteboard to rule out some diseases and account for any new symptoms. And on and on until the dramatic final "A-ha!" moment.

Stylized though it might be, House does at its core capture the guess-and-check nature of most diagnoses in medicine today. But rapid, highly sensitive DNA testing is poised to change that.

Scientists at the University of California, San Francisco have announced a DNA-based diagnostic technique built around what they call Next-Generation Sequencing. Doctors take a sample of blood or other bodily fluid and use the new sequencing technology to tease out every bit of genetic material present.

"As opposed to the way we normally diagnose infectious disease — meaning we target a single infectious agent at a time — this test works by detecting all the DNA present in clinical samples," study-runner Charles Chiu told NPR's Richard Harris.

Chiu and his colleagues broke new ground by saving the life of a young boy. The results are reported in the New England Journal of Medicine, and science journalist Carl Zimmer covered the story for the New York Times:

> Joshua Osborn, 14, lay in a coma at American Family Children's Hospital in Madison, Wis. For weeks his brain had been swelling with fluid, and a battery of tests had failed to reveal the cause.
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> The doctors told his parents, Clark and Julie, that they wanted to run one more test with an experimental new technology. Scientists would search Joshua's cerebrospinal fluid for pieces of DNA. Some of them might belong to the pathogen causing his encephalitis.
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> The Osborns agreed, although they were skeptical that the test would succeed where so many others had failed. But in the first procedure of its kind, researchers at the University of California, San Francisco, managed to pinpoint the cause of Joshua's problem — within 48 hours. He had been infected with an obscure species of bacteria. Once identified, it was eradicated within days.

At NPR, Harris adds Andrea Struve's experience to Chiu's list of case-studies (this one published in Genome Research):

> One of their early patients is Andrea Struve, a 21-year-old San Franciscan who returned from

> 40 days in the Australian Outback last year with a nasty set of symptoms.
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> "I was in classes, sweating profusely with a fever and joint pain, and it just wasn't fun, so that's when I went to the doctor," she says.
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> Her doctor made a bunch of educated guesses about the underlying cause, but all the tests came back negative. So physicians enrolled Struve in a study at UC San Francisco to try out a different approach.
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> […] It turns out that she was infected with a virus related to chicken pox — one that normally causes a roseola rash in young children.
> "They're not entirely sure why I got it," Struve says, adding that she was "really, really glad it was something that would be gone in a month instead of six months to a year."

Both cases represent a turning point in the application of genetic analysis in medicine. True, genetic tests have been used for years now to identify pathogens or, even more frequently, to identify harmful mutations or risk factors in individuals. Never has the technology behind sequencing been efficient enough, however, to work as a tool in a medical emergency. Zimmer captures the excitement:

> "This is an absolutely great story — it's a tremendous tour de force," said Tom Slezak, the leader of the pathogen informatics team at the Lawrence Livermore National Laboratory, who was not involved in the study.

It all comes down to time. In the hour-long episode of House, the diagnosis is almost never made until the last moments of the show as the patient-of-the-week is teetering on the brink. That fiction underscores an important real life point: just trying to figure out what's wrong can be the most involved part of the treatment process. A master test that can eliminate guesswork and save precious time would be a boon indeed.

But there are several limitations to consider. Any such test is only as good as the database against which the results are compared: if we don't have advanced knowledge of what the DNA of a particular pathogen looks like, finding bits of its DNA will be of limited use. Thankfully, our database of genetic information on ourselves and our pathogens is only going to become more comprehensive as time goes on. Yet as it grows it becomes increasingly clear that we are veritable zoos of microbial life.

Harris points out a sobering reality of Chiu's tests:

> So far Chiu has tested this technology in more than 30 cases. He says he and his colleagues have been able to identify an infectious culprit about 25 percent of the time. Some of the remaining cases turned out not to be infections at all, Chiu says.

A twenty-five percent success rate is certain to be helpful, but it's a long way off from an all-powerful test. Furthermore, both of these tour de force case studies are best-case scenarios. Both patients had

mysterious illnesses, and in both cases they were afflicted with relatively easy-to-kill pathogens. What if it was a more obscure virus? A drug-resistant bacteria? Something altogether different like the single-celled blood parasite that causes malaria? It's unfortunate, but a successful diagnosis does not always mean a successful cure.

As things stand, Harris writes:

> The test is still experimental. It's done in a university setting and each run costs about $1,000 in labor and materials, not counting the expensive machinery. But the price of this technology continues to drop. Chiu hopes in time it will become the go-to analysis when traditional blood tests don't provide the answer.

Again, time is of the essence: though in this case, time seems to be in Chiu's side. As genetic databases grow, technology speeds up, and the costs drop it seems very likely that something like Chiu's dream of a comprehensive test may someday become a reality.

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Additional Resources:

- "Bioinformatics and epigenetics – computer-aided cancer diagnosis," Nils Ehrenberg | Medical Xpress
- "Patient Cracks Her Own Mysterious Dual Diagnosis," Ron Zimmerman | MedScape
- "Deeper into genetic challenges of psychiatric diagnosis," Vaughan Bell | Mind Hacks