New urine test could reduce need for blood samples

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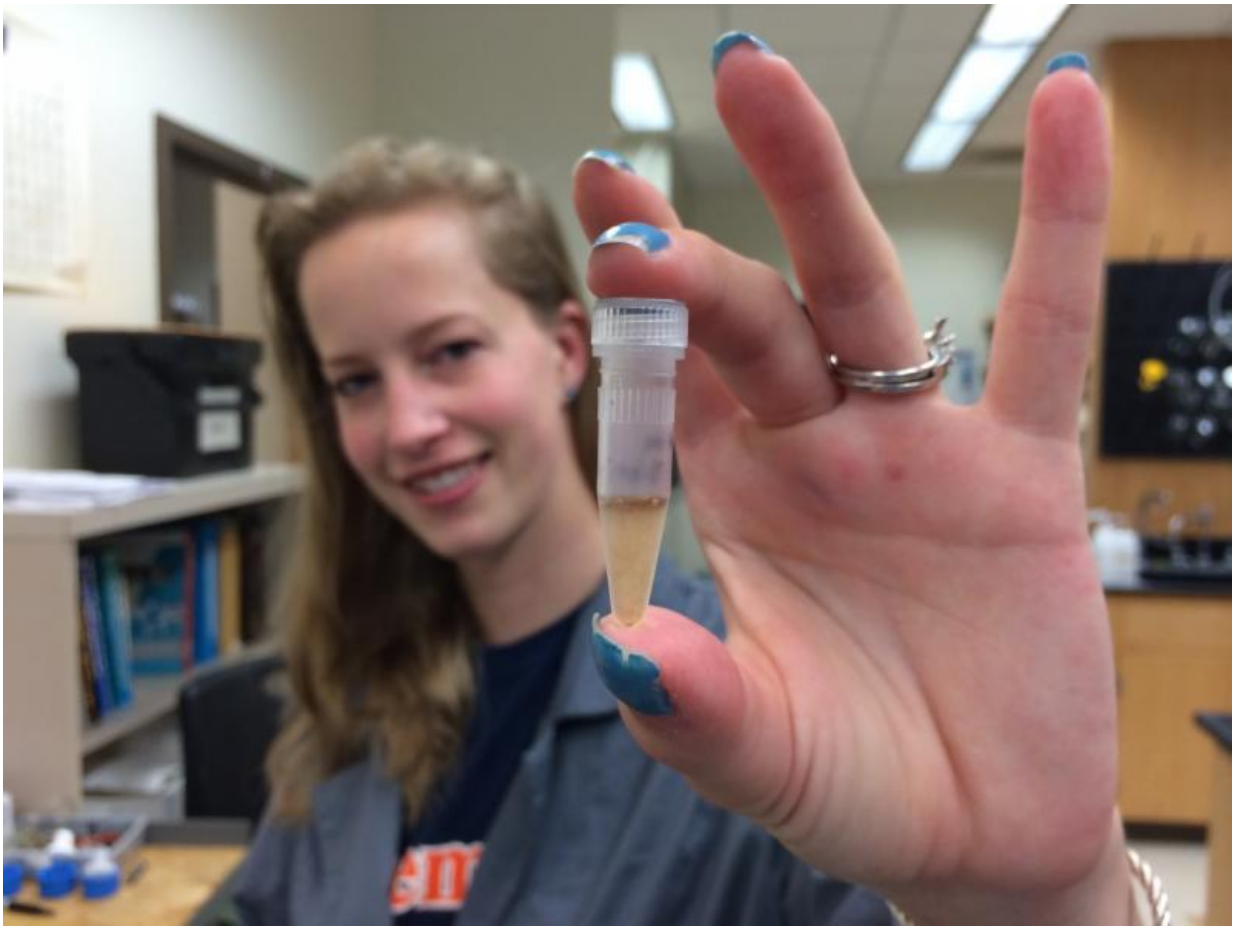

Pierson holds a vial of proteins to be extracted for analysis.

If you've been to the doctor, you probably know what to do when you're

handed a plastic cup and shown to the bathroom.

Most patients hand over the sample and give little thought to what happens when it's shipped to the lab for analysis.

Ken Marcus and his students are the exceptions. They have developed a new testing method that they believe will reduce costs, get faster results and lower the volume of urine needed for a sample.

Marissa Pierson, a master's student, closes the lid on a centrifuge while working in a Clemson University lab with Ken Marcus.

It's great news for patients who get the willies when the nurse pulls out the needle to draw blood. The method Marcus and his students have developed could help make it possible to use urine instead of blood to test for more diseases.

Proteins in urine, for example, could help detect early signs of coronary heart disease, tell whether the body is rejecting a transplanted kidney and show whether someone has sleeping sickness.

The trouble with testing urine is that it's awash in salt, Marcus said. It can be tricky to isolate the proteins that act as biomarkers. Those biomarkers are the clues that tell whether the patient is sick or has ingested a drug.

"You've got almost seawater coming out of you, and I'm trying to find something far smaller than a needle in a haystack," Marcus said. "The concentrations of these proteins would be one part in a billion."

The magic ingredient in the group's research looks like kite string, but it's no ordinary twine. It's made of capillary-channeled polymer fibers.

As part of a study, Marcus and his students packed the fibers into plastic tubes and then passed urine samples through the tubes by spinning them in a centrifuge for 30 seconds. Then the researchers ran de-ionized water through the tubes for a minute to wash off salt and other contaminants.

Proteins are hydrophobic, so they remained stuck to the fibers. Researchers extracted the proteins by running a solvent through the tubes in the centrifuge for 30 seconds.

When it was all done, researchers were left with purified proteins that could be stored in a plastic vial and refrigerated until it's time for testing. The team was able to extract 12 samples in about five minutes, limited only by centrifuge capacity.

In urine tests commonly used now, polymer beads extract the proteins.

"The difference is that ours is smaller, faster and cheaper," Marcus said.

The team's work was recently published by the journal *Proteomics-Clinical Applications*. The authors were Benjamin T. Manard, Sarah M.H. Jones and Marcus.

In a review of the article, professor Youhe Gao of Beijing Normal University wrote that the method "may have more profound implications than the authors have claimed, simply because urine is more important than most biomarker researchers realize."

Various mechanisms in the body limit changes in the blood. Urine, however, "accumulates all kinds of changes and should be a better biomarker source than blood," Gao wrote.

Further, urine is the mostly easily obtained bodily fluid, he wrote. Sweat can also be obtained noninvasively, but it may not be as informative and

free of contaminants as urine, Gao wrote.

The new method should also make it easier to test urine samples from babies, Marcus said. One of the challenges now is getting a large enough sample. But the new method requires only a few microliters of urine.

The research has been about a decade in the making with various students working on it over the years. They include W. Clay Davis, Dwella M. Nelson, Brad Knippel, Rayman D. Stannelle, Christine M. Straut, Tim M. Brewer, Jennifer J. Pittman, Manoj Randunu, Benjamin T. Manard, Carolyn Q. Burdette and Abby J. Schadock-Hewitt.

For Marcus, the most important thing is to create a research environment that produces well-prepared graduates.

Marcus, a professor of analytical chemistry, said that he has graduated 33 Ph.D. students with more than half going on to work for national labs. Others work in industry and for the Centers for Disease Control and Prevention. Still others in his lab are focused on the development of analytical methods for post-detonation nuclear forensics.

"My pride is putting those people out and seeing them get really good jobs," Marcus said.

Marissa Pierson, a master's student from Pittsburgh, is among the latest crop of students to work in Marcus' lab. On a recent morning, she was putting samples in the centrifuge.

"It's a really awesome place to work," she said. "It's good to know that people will use this."

Karl Dieter, the chair of the chemistry department, said it is heartening to see a professor so passionate about his students.

"Ken has created scientists that have gone on to exciting, impressive careers," Dieter said. "When they graduate, they are ready to get some of the most desirable jobs in their field and contribute immediately."

More information: *Proteomics-Clinical Applications*, onlinelibrary.wiley.com/doi/10 … a.201400081/abstract

Provided by Clemson University