Stacking the Odds to Detect Dengue from Saliva

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Saliva-based tests are not new. The Chinese used saliva for an ancient polygraph called the rice test, in which an interrogator placed grains of dry rice into the mouth of a suspect and asked the suspect to spit out the rice. If the grains of rice stuck to the mouth of the suspect, stress-induced xerostomia was assumed—and therefore the suspect was guilty.

Fortunately, over the past 3000 years, we have made progress in salivary diagnostics. One of the latest advances is the design of a new paper-based immunoassay by Jackie Y. Ying, executive director of the Institute of Bioengineering and Nanotechnology in Singapore.

Why Is This Innovation Important?

Jackie Y. Ying

The home pregnancy test is an example of a traditional paper-based immunoassay, known as the lateral flow test. In such a test, the sample flows laterally through the test strip by capillary action. The sample comes in contact with particles with which it can react as it migrates through the strip. This design is popular because of its simplicity and low cost.

However, although saliva is a rich source of biomarkers, its use in rapid tests has been complicated by proteinaceous products in saliva that interfere with lateral flow reactions. According to George Whitesides of Harvard University, "the basic lateral flow immunoassay format is industry standard, but typically not for saliva (primarily because the concentration of analytes in saliva is very variable, since the volume of saliva depends on state of hydration, hunger, et cetera)."

In response to this need, Ying and colleagues developed a new immunoassay called the stacking flow assay. "We feel it is necessary to separate the saliva sample and reagent into 2 separate paths to reduce the nonspecific background and obtain a clear result," explains Ying. "In order to achieve uniform flow with 2 input paths, we have come up with the stacking flow design."

"The innovation in this system is that it has been designed to work with saliva (a good idea, if the quantitative concentration of biomarker is not important, but only binary yes/no results are sufficient), and that the system has been designed to remove interfering materials present in saliva," remarks Whitesides. "This is a bioengineering problem, and actually a very difficult one."

However, he adds a level of caution. "This test—like most lateral flow/rapid immunoassays—is unlikely to be very useful in tests where a quantitative assay is required, such as management of HIV (where HIV biomarkers during antiretroviral therapy are in very low concentrations in blood) or classification of drug resistance in pathogens."

Ying chose to demonstrate the mechanism of her technology to detect antibodies to dengue *(1)*. "According to the US Centers for Disease Control and Prevention, dengue is a leading cause of death and illness in tropical and subtropical countries," says Ying. "In Singapore, the number of dengue cases is alarming."

How Does It Work?

George Whitesides

To keep the test user friendly, Ying wanted to avoid sample pretreatment or multiple steps at specific time points. She accomplished this through a novel stacking of flow paths that mix at the distal end of the test strip. The flow path for the sample is made of fiberglass matrix that removes protein and other particulates from the sample to reduce nonspecific binding. Above the flow path is a liquid impermeable flow regulator overlaid with a glass fiber reagent flow path. The flow regulator moderates the mixing between the paths to give even output lines.

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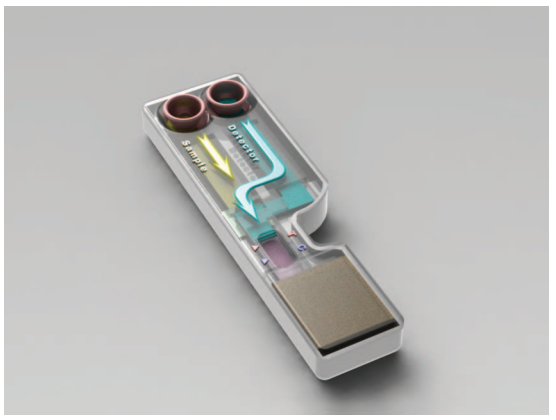

Fig. 1. Three-dimensional model of the Institute of Bioengineering and Nanotechnology's rapid test kit that detects dengue-specific antibodies from saliva. Arrows indicate the sample and detector flow paths.

The design uses standard nitrocellulose for the test strip. The layers stack into a cassette (Fig. 1) that has 2 reservoirs for application of the sample and reagents. Because the biomarker signals in saliva are relatively low, large sample volumes need to be applied to the test and can be held in the designated reservoir.

"Our competitors for saliva testing use conventional lateral flow devices," says Ying. "Compared to these conventional lateral flow test strips, our stacking flow design is able to detect targets directly from a large volume of saliva sample."

In her demonstration, Ying lays and dries type 2 dengue antigens as the test line onto the test strip. Protein G–conjugated 40-nm gold nanoparticles, which serve as the detector conjugates, are added to the sample before the test. When applied, the sample and reagents run through their respective paths. As in standard immunoassays, dengue-IgG conjugate complexes are captured by the fixed dengue antigens, forming a visible test line if present.

Ying has shown the mixing of up to 3 flow paths and claims that more paths can be added at the trade-off of a longer test strip, and thereby larger device size.

Where Can This Technology Fit in the Laboratory?

The IgGs detected by Ying's test are present only in a secondary dengue infection. "It is believed that secondary dengue infection is more likely to develop into more serious conditions, such as dengue shock syndrome and dengue hemorrhagic fever," she says. "Therefore, it would be important to identify these patients early so that they can have access to the appropriate treatment and care."

Whitesides is cautious. "The problem is whether this is important. Dengue hemorrhagic fever is a serious and often fatal disease. I'm not sure how the information provided by the test would be used by clinicians. There is no treatment for dengue, and so the question is what do you do—as a clinician—if the test is positive? And if it's a false positive, can you do harm by intervening?"

What Would Whitesides Do Next?

"One generally has no idea if these sorts of tests are of any real value unless they have been (successfully) through field trials—say, 1000 patients in the environment in which they would actually be used," Whitesides says. "Laboratory results are interesting, but not good indicators of a successful field trial, and certainly no indication that the laboratory test will ever make it to market."

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Reference

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