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Sink or swim: using density as a signal for quantitative immunoassays

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Immunoassays are powerful tools for detecting specific molecular targets from complex samples. The first immunoassays, developed in the 1950s, used radioactively labeled antigens (which specifically bind to analytes of interest) to measure the concentration of insulin in plasma. Due to the radioactive signals, these tests were confined to sophisticated laboratories and required highly trained personnel, special licensing, and expensive equipment. Many efforts have since been made to simplify immunoassays and develop portable, point-of-care (POC) testing formats. For example, one of the most well-known immunoassays, the pregnancy strip, uses dye molecules, rather than radioactive isotopes, to report on the presence of a specific hormone found in the urine of a pregnant woman. Colorimetric signals offer a convenient

approach to perform qualitative measurements; however, these readouts are subjective, and it can be difficult to extract quantitative information (e.g., the exact amount of hormone, in mIU mL⁻¹) from them. Other signals, such as fluorescence, chemiluminescence, and electrochemical, can be used to obtain more quantitative results; however, they require electronics and signal processing equipment, rendering them inconvenient for POC applications. In this regard, it is important to develop new signals that can be quantified without the need of specialized equipment for POC tests.

In this issue of *Lab on a Chip*, Whitesides *et al.* (DOI: 10.1039/C4LC01161A) present their efforts on developing new signals for POC immunoassays. They used density as a measurable target to reflect the quantity of molecular analytes in solution. Therefore, they demonstrated immunoassays that can be read using only the naked eye and a ruler. The use of

density to identify objects can be dated back to the third century BC, when Archimedes used density as a marker to determine the purity of King Hiero's new crown. Whitesides *et al.* revitalize the role of this old parameter in analysis by introducing the Magnetic Levitation (MagLev) method for high-sensitivity density determination. By placing two permanent magnets coaxially facing each other, the density of objects can be obtained by observing the 'levitation height' of the object. Binding of analytes is designed to occur on the surface of polystyrene beads and the signal is amplified by gold-labeled antibody and electroless gold or silver deposition. As a result, the presence and the amount of target analytes will result in a change in density of the polystyrene beads, which is reported as a change in the levitation height of the bead. This platform is demonstrated for both competitive and indirect immunoassays through probing neomycin in a milk sample and Hepatitis C virus NS3 protein and syphilis *T. pallidum* p47 protein in serum, respectively.

This new readout strategy minimizes the requirements of external resources to measure an immunoassay. It does not need any complex equipment or electricity to achieve accurate quantitative measurements. Thus it is ideal for POC applications in remote sites and resource-poor areas. For future development, if this technique allows convenient and accurate quantification of target analytes, many of the current POC immunoassays can be further simplified and improved, and made more amenable to portable applications.

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