

Low-Cost Agglutination Assays for Medical Diagnostic Applications Using a Whole-Cell Biosensor with Tunable Limit of Detection

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EDITORIAL

Whole-cell biosensors have the potential to be the foundation for low-cost, easy-to-use diagnostic tests that can be quickly deployed for Point-of-Care (POC) testing, but detecting analytes such as proteins that cannot easily diffuse across the cell membrane has proven difficult to date. Using an *E. coli* whole-cell biosensor surface-displaying nanobodies that bind specifically to a target protein analyte, we built a novel biosensing platform based on cell agglutination. We demonstrate the practicality of this architecture by detecting a simulated analyte at nanomolar quantities as a proof-of-concept. Furthermore, we demonstrate the flexibility of the design architecture by developing assays that can detect a wide variety of model analyte concentrations using simple design principles and a mathematical model. Finally, we re-engineer our whole-cell biosensor to detect a medically important biomarker by displaying two distinct nanobodies against human fibrinogen in diluted human plasma, demonstrating a detection limit as low as 10 pM. Overall, we show that our agglutination technique satisfies POC testing requirements by combining low-cost nanobody production, configurable detection range, and low detection limits. This technique offers the potential to generate low-cost diagnostics for use in underdeveloped countries, as well as for routine medical testing and personalized medication. One of the most promising biotechnologies for improving global health is affordable point-of-testing diagnostic technology applied to resource-limited settings. Scientific obstacles, economic constraints, and practical issues must all be considered in the development of new diagnostics and their successful implementation in the field by end users. High-impact technology will allow for sensitive and specific detection, will be low-cost and portable to assure accessibility, and will be

designed in a user-friendly manner that does not require specialised equipment. In vitro diagnostics, immunoassays are the most often used technology. Antibodies for the target analyte can be made with excellent specificity and relative ease. Latex Agglutination Tests (LAT), which use antibody molecules immobilised on latex particles to detect the presence of an analyte, are available for more than 300 diseases and biomolecules among the numerous types of immunoassay formats for the creation of quick diagnostic tests. Multivalent immuno-latex particles can detect and build higher-order complexes with their target analyte molecules through particular interactions. Extensive cross-linking between analyte molecules and latex particles occurs during the agglutination reaction, resulting in the creation of very large complexes that can be seen with the naked eye or monitored spectrophotometrically.

The utilization of entire cells as a bio analytical platform for in vitro medical diagnostics has several advantages, including inexpensive production costs. Whole cells can self-replicate and produce recognition elements like antibodies, obviating the need for costly purifying methods. Whole cells can also provide physiologically relevant data on the bioavailability of the analyte because they are living organisms. Bacterial whole-cell biosensors have been genetically designed to detect medically relevant analytes in both serum and urine samples, including metabolites, hazardous compounds, and mercury in urine, hydroxylated polychlorinated biphenyls in serum, and nitrogen oxides in both serum and urine samples. Whole-cell biosensors typically rely on intracellular detection of the target analyte, however this approach is limited to detecting analytes that can diffuse or be actively transported across the bacterial cell membrane. Nonetheless, many medically important analytes, such as protein biomarkers for disease states, are unable to permeate the membrane barrier.

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