# Development of a Multiplex fluorescence and colour change HIV/TB Diagnostic Assay Based on the Microarray Technology

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**Background**: HIV/AIDS mortality is caused by opportunistic illnesses/infections. In Africa, these include infections by Mycobacterium tuberculosis (*M.tb*) responsible for tuberculosis (TB). HIV co-infection with *M.tb* has negative implications for disease management given that each pathogen accelerates the morbidity caused by the other. The effective management of patients infected with HIV/TB is restricted by the fact that their diagnosis is done separately. The situation is more difficult in remote areas where patients must wait for much longer to obtain their TB diagnostic results. In addition, the current diagnostic tests for the detection of TB such as chest X-ray and bacterial culture have a long turnaround time, are expensive to perform, and require sophisticated equipment and trained personnel. It is in this context that this project sought to develop a point-of-care (POC) HIV/TB multiplex microarray-based technology for the fast detection of HIV/TB using one test.

**Method**: The project used epoxy-coated glass slides and high-binding 96 well plates to which HIV-1 p24 and *M.tb* CFP10, ESAT6 and pstS1 antigens were immobilized. The antigens were incubated with anti-p24, anti-CFP10, anti-ESAT6 and anti-pstS1 primary antibodies diluted in human serum. Detection was achieved by means of Alexa fluor and horseradish peroxidase conjugated secondary antibodies for fluorescence and colour change detection respectively.

**Results**: Data showed that the HIV and TB antigen-antibody reactions were sensitive and specific in both fluorescence and colour change detections. The limit of detection

was determined to be 0.954 ng/ml with the epoxy-coated glass slides and 4474.6 ng/ml for the 96 well high binding plates. This showed that the epoxy-glass slides were more sensitive to the HIV/TB antibody antigen reactions. In addition, the limit of detection concentrations in this study were lower than the reported physiological concentrations of HIV/TB antibodies in infected patients. In addition to the sensitivity and specificity studies conducted, the technology stability studies were also evaluated, and we showed that the HIV/TB antigens on the slides could be stored dry at room temperature for 90 days.

**Conclusion**: Overall, the data showed that the HIV/TB multiplex microarray-based technology can potentially be used as a fast, user friendly and cheap technology for the detection of the two diseases simultaneously at POC. This is imperative given that HIV and TB are diseases affecting mostly poor and developing countries in the world.