

## Multiplexed Immunoassays by Flow Cytometry: Relevance to Diagnosis and Surveillance of Infectious Diseases in Resource-poor Settings

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An accurate rapid diagnosis is the cornerstone of efficient clinical and epidemiological management of infections. Here we discuss the relevance of an emerging technology, the multiplexed immunoassays by flow cytometry, for the diagnosis of infectious diseases. In these assays, multiple fluorescent microspheres, conjugated to antigens or antibodies, constitute the platform for the molecular interactions. This is a potentially inexpensive technology, because at its minimal configuration it can run, battery operated, on a single red diode laser. The bead based assays are more sensitive than traditional immunoassays, have a high throughput capacity and provide a high analytical dynamic range. Additionally, they have multiplexing ability, i.e., are capable of measuring multiple antibodies or antigens simultaneously. This latter property opens an unprecedented possibility of arranging combined tests designed to answer questions of differential diagnosis.

In our paper five different areas where multiplexed immunoassays by flow cytometry can make an impact in resource-poor settings are discussed: (i) Infections causing rash and fever in children. This is particularly important in the context of WHO's measles control and elimination programmes; (ii) Sero-epidemiological studies on vaccine preventable diseases, which aim at evaluating the vaccination process under the Expanded Programme on Immunisation (EPI); (iii, iv) Genital ulcers and vaginal discharge, both requiring more precise differential diagnosis for effective therapy – which also aids to curb the spread of HIV infection; and finally (v) In blood banking, where busy laboratories would benefit enormously from universal multiplexed antibody assays for HIV, hepatitis C, HTLV and syphilis.

This new area of technology can also be developed to serve a number of other diagnostic needs, using the same or similar inexpensive equipment, such as monitoring CD4 counts and viral load in HIV-infected patients as well as detecting malaria parasites in the blood. Once these tests are handled by the same technique and reported at the same time – they are likely to greatly assist patient management and become practical tools, particularly in the current demand for economical monitoring of the recently introduced anti-retroviral therapy in resource-poor settings.

Efficient clinical management and epidemiological surveillance of infectious diseases depend upon an accurate diagnosis [1, 2]. In most clinical and surveillance laboratories of the developing world this process relies on serological techniques aimed at the detection of pathogen specific antibodies. These are frequently measured with enzyme-linked immunosorbent assays (ELISA). These tests' popularity is due to their high sensitivity and specificity, good reproducibility and a high throughput capacity with affordable costs [3, 4]. However, ELISA's require trained personnel and some specific equipment, and are rarely suitable for field conditions in resource-poor settings [5].

The need for field-testing devices in developing countries is well illustrated by the success of simple tests, performed by technicians with basic training and interpreted visually, for the detection of antibodies to human immunodeficiency virus

(HIV) [6, 7]. With these simple and rapid tests, it has been possible to extend the diagnosis of HIV infection to less equipped laboratories outside major centres. Moreover, with results available on the same day, these assays now constitute an important component of volunteer testing clinics [8]. Nevertheless, even these simple assays are not ideal; their weaknesses include a lower sensitivity when compared to ELISA [9, 10], difficulties in interpreting weakly positive sera [11], a high cost [12] and low throughput capacity. Among the features desirable for an ideal assay some are compatible and others tend to be mutually exclusive (Table 1). On one hand, the most obvious dilemma is that a sophisticated assay is unlikely to be cost-effective and/or suited to field conditions. On the other hand, field tests may remain short of the standard performance required in the central laboratories [9-12].