Protein Analysis by Multiplex Techniques

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Screening of biomarkers by high throughput (HT) techniques has great potential for clinical and genetic analysis and medical diagnostics. The reason being that proteins can specify the state of disease progress and the functions of normal biological processes within the human body, HT screening techniques that recognize proteins and their expression levels are very essential for early detection, diagnosis, and finally therapy.

The most widely used method for protein analysis in basic research and clinical diagnostics is enzyme-linked immunosorbent assay (ELISA). Though mass spectrometry can be used for protein analysis, it has its own limitations. For example, it can only be used to analyze one or a few samples at a time, and is not suitable for high-throughput assays with small assay volumes. Considerable interest has been drawn to the development of protein-based multiplex detection techniques. One of the important assays developed is the microbead-based multiplexed assay for analyzing the types and concentrations of target molecules in an "unknown" solution. Target molecules could be antigens, antibodies, oligonucleotides, receptors, peptides, or enzyme substrates and may be labeled with a reporting molecule (e.g. a fluorescent molecule). This assay can be used in terms of proteomics: Protein profiling (multiplexing cytokine assays), protein function and interaction (receptor-ligand binding like enzyme activity, protein-protein interaction; antibody cross-reactivity and specificity; epitope mapping), post-translational modification (signal transduction proteins) and also in terms of molecular diagnostics (SNP genotyping, detection of disease-related mutations); multiplex diagnostic immunoassays (blood banking, infectious disease testing, vaccine efficacy testing, autoimmune testing, allergy testing and biomarker detection).

In the beginning of the 21st Century, tuberculosis (TB) was declared as a Global Emergency by World Health Organization (WHO). TB remains one of the major public health concerns in the South-East Asia WHO Region. Pakistan now ranks 5th amongst TB high-burden countries worldwide and is also estimated to have the fourth highest prevalence of multidrug resistant TB (MDR-TB). The coughing and sneezing of patients causes spread of disease due to the generation of infectious aerosols. This route of infection in human population requires early diagnosis of TB to eradicate and limit the disease. The ideal test for TB diagnosis must be able to detect early infection with high sensitivity and specificity, must generate results rapidly, be inexpensive and cause little or no patient discomfort. At least one of the above requirements are not fulfilled by the presently available diagnostic tests such as sputum smear microscopic examination, culturing of sputum samples, PCR-based diagnosis, lipoarabinomannan detection in sera, Mantoux test and chest X-ray. Thus we have made efforts to develop and optimize a multiplex immunoassay system for detecting antibodies and cell-based immune responses to *Mycobacterium tuberculosis* (*M. tb.*) and for detecting *M. tb.* antigens in Pakistani TB patients with the boost in recombinant DNA technology.