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# Application of Flow-Cytometry In a Field Of Microbiology; A New Horizon For Rapid Microbial Diagnosis

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## ABSTRACT

Diagnostics are the backbone for the provision of good quality health care facilities to community. With the innovation in science and technology, many new modalities are coming up having certain benefits and limitations. Though in microbiology culture and sensitivity is still a gold standard but with certain limitation like increased time consumption, less automation, deficiency for the provision of microbial proteiomic and genomic details. Therefore, in era of increased anti microbial resistance, the scenario is urging for the availability of novel, automated, rapid, cost effective, and more sensitive strategies to combat the situation. Therefore the objectives of this review article was to provide an evidence regarding application of flow cytometry in a field of microbiology. It is concluded that flow cytometric method is a rapid, accurate and more sensitive method for simultaneous identification and quantification of microbial physiological and compositional states



## Keywords

Flow-cytometry, Microbiology, Microbial Diagnosis, Sensitivity And Specificity Of Flow Cytometry, Anti Microbial Resistance, Proteomic Analysis, Genomic Analysis


## INTRODUCTION

The encroachments in science and technology is opening up new horizons for better health care opportunities. Diagnostics are the backbone for any such innovation. Novel diagnostic approaches are contributing for the provision of more sensitive and specific results to provide share for good health care. Flow cytometry (FCM) is one of such sophisticated techniques well known for decades regarding it's utility for proteiomic analysis. But now it's utility is coming up in the field of microbiology to identify microbes with similar population. It works on a principle of light scattering and fluorescence emission by the specific fluorescently labelled probes and cells, upon their passage through a laser beam. [1]

A high throughput, rapid proceedings, quantitative evaluation and multi-parametric analysis of cell populations even at a single cell level are the biggest advantages of FCM. Moreover, based upon size, shape, complexity, and ability to fluorescence measurements at multiple wavelengths, it assists physical sorting to further subpopulations. This helps better comprehension for identifying fluorescently labelled antibodies, fluorescent proteins, DNA binding dyes, viability dyes, and ion indicator dyes. The further advance advancements in traditional FCM includes imaging FCM, mass cytometry and Raman FCM, having added sensitivity and specificity even to analyze 30-50 or more parameters on a single cell. [2]

In microbiology, culture and sensitivity is still a gold standard. Along with it's few limitations to rapidly identify microbe and it's drug susceptibility plus an immensely added Global burden of anti-microbial resistance, is urging for the availability of novel and authentic diagnostic modality. The evolution of techniques and technologies over the past two decades, like recombinant technology, next-generation sequencing, nucleic acid sequence based assays, Sanger sequencing, metagenomics sequencing, multiplex PCR via microarray, etc are contributing with certain pros and cons to enhance health care facilities. [3]

## LITERATURE REVIEW

In view of Global health statistics, gastro intestinal infections are having the highest prevalence amongst adolescent and adult age groups. [3] The human gut holds billions of microorganisms, collectively referred to as the microbiota. These are considered to play important roles in human health and disease. [4] About 50% of the bacteria comprising the human gut microbiota lack a complete reference genome to study their disease pattern. [3,4] Here comes a limitation with a gold standard culture and sensitivity used in microbiology. By which microbe and drug susceptibility can be identified but not the genomic and proteomic details of microbe responsible for disease. Besides this even cultivating few members of microbiota is still a challenging task for health care providers. [5] Especially when things be narrowing down and coming at a level of differentiation at species and strains in complex genomes.[6,7]

Even taking in consideration the metagenomic sequencing, helps estimation of 3 million unique predicted genes in the microbiota of gut. But the easy availability and lack of skills to handle such delicate approaches comes in it's limitation. [8-10] Further elaborating metagenomics-based approaches reveal the functional potential of microbial communities. They are less sensitive to appropriately link genetic features i.e bacterial genes and mobile genetic elements, amongst each other and to main bacterial genomes. Their inadequacy to capture bacterial microdiversity based upon genus, species/strain within complex communities is an added limitation. Somewhat utilizing fluorescence-activated cell sorting and flow cytometric separation even uptil a level of single bacterial cells from the human microbiota is filling up the gap. It helps to differentiate between functional potential and variation for diverse elements between individual microbes in the human microbiota. [11]

The microfluidic flow cytometric approach was well introduced for human and eukaryotic cells identification. It helps isolation of even single microbial cells from the samples. This is comparable with a traditional flow cytometry which generally targets the populations of cells with specific properties, and have focused less on capturing single cells. The limitation is

better covered by microfluidics flow cytometry, which is less expensive, simpler, easily handled and more autonomous alternative to conventional flow cytometers. The point-of-care diagnostics is one of the added benefits for its on-site analyses. [12]

Besides well-known use of blood samples, stool sample can also be used to study relationships between bacteria and bacteriophages in the human gut via microfluidics flow cytometry. [13-15] One more published study emphasized utilization of flow cytometry and cell sorting to isolate, separate, and cultivate new strains for commensal of fecal material. They have focused aerobic Faecalibacterium prausnitzii species and Christensenella minuta species for said evidence. It was also identified that targeted cell-sorting under anaerobic conditions is a promising tool for the study of fecal microbiota. Identification of proteiomic details using specific antibodies had also proven successful outcome. [16]

A published report by *Dossou N etal*, in Journal of Microbiology Spectrum concluded that biological processing can also done on flow-cytometry. He emphasized that analysis and interpretation of these fluids plays a significant role in early diagnosing and hence managing the disease. Despite a well-known gold standard i.e manual counting chambers, Gram/ ZN staining and cultures, certain limitation with identified upon comparative analysis with flow cytometry. The predictive values for cell counts were found higher in culture and direct Gram stain positive specimens. Hence concluded that flow cytometry can be used to upstream cytological and microbiological routine procedures for rapid and accurate and diagnosis of infection in biological fluid samples. [17]

Another study by *Allain M etal* in 2019 had provided a strong evidence for the good utility of flowcytometry to study urine analysis, when compared to optical microscopy and culture. The results showed that microscopy/flow cytometry discrepancy rate was 8.5% for WBCs, and 16% for RBCs. The majority of these discrepancies corresponded to quantities close to the clinical threshold, mostly higher by automatic than by microscopic counts. Moreover a good sensitivity was observed for the identification of population groups of Gram positive and Gram negative bacilli, when compared with culture results. [18]

The usage of blood sample was supported by another published study in which flow cytometry assay for intracellular cytokine staining (FC-ICS) was introduced. The FC-ICS was compared with a commercially-available cytokine release assay (the QuantiFERON® SARS-CoV-2 Test [QF]) for detection and quantification of SARS-CoV-2-Spike (S)-reactive-IFN-γ-producing T cells after COVID-19 vaccination. The results showed that a greater sensitivity for FC-ICS assay upon QF test. However it was clarified that a caution showed be taken when equating SARS-CoV-2 T-cell immune responses using diverse analytical platforms. [19,20]

## Conclusion

It is concluded that flow cytometric method is a rapid, accurate and more sensitive method for simultaneous identification and quantification of microbial physiological and compositional states

## RECOMMENDATIONS

1. The microbiologist, molecular genetic specialists and researchers should join hands to do do extensive original prospective research to provide strong evidence for flowcytemtic evaluation in microbiology. This is in view of limited original research data focusing it's utility
2. The microbiologist, molecular genetic specialists and researchers should join hands to utilize flow cytometry modality focusing simultaneously identification of genomic and proteiomic details.
3. In view of increase emergence of anti microbial resistance, there is dire need for early availability of this test as part of routine microbiological testing so that time accurate management can be started.

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