BJMG 28 (1), 2025 • 1-6
DOI: 10.2478/bjmg-2025-00014
sciendo
ORIGINAL ARTICLE

FLOW CYTOMETRY MULTIPLEX BEAD ARRAY TECHNOLOGY AND ITS IMMUNOLOGICAL CLINICAL APPLICATIONS IN COVID-19 ERA

Pavlovski I[1], Riachi EM[2], Macha S[3], Atanasova-Pancevska N[1]

[*]**Corresponding Author:** Igor Pavlovski, Department of Microbiology and Microbial Biotechnology Institute of Biology, Faculty of Natural Sciences and Mathematics «Sts Cyril and Methodius» University, 1000 Skopje, North Macedonia, phone number: +38970276525, E-mail: ipavlovski777@gmail.com

ABSTRACT

A thorough functional evaluation of the immune system requires a method capable of measuring multiple cytokines to assess the adaptive immune response, as well as immunoglobulin isotypes and seroconversion to evaluate the humoral immune response following infections and vaccinations. Flow Cytometry Multiplex Bead Array (FCMBA) technology has been shown to be highly effective in detecting and quantifying numerous analytes simultaneously in a single reaction well. This technique is increasingly being used in immune profiling for cytokines in various disease contexts, as well as in adoptive cell transfer, immunotherapies, and more recently, in the characterization of humoral responses to newly developed COVID-19 vaccinations. FCMBA technology has shown advantages in assessing the depth of the humoral response mounted against COVID-19 vaccinations, compared to post-COVID infection status, and screening for the suitability of sera for use in COVID-19 convalescent plasma therapy. In this article, we present an overview of the FCMBA technology concept and its versatility, highlighting its ability to map many essential analytes and its superiority over conventional Enzyme Linked Immunosorbent Assay (ELISA) techniques. Furthermore, we discuss the current and potential clinical applications of this technology in deciphering COVID-19-triggered humoral immune responses, whether after contracting the infection or post-vaccination.

Keywords: Flow Cytometry Multiplex Bead Array, COVID-19, Serology, Immunology

INTRODUCTION

Coronavirus disease 2019 (COVID-19), the infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first identified in December 2019 in Wuhan, China, and has spread rapidly around the world [1]). Most people infected with SARS-CoV-2 display an antibody response approximately one week after the infection [2].

Rapid and accurate serological tests detecting anti-SARS-CoV-2 antibodies are essential for estimating SARS-CoV-2 seroprevalence and differentiating immune response to natural infection from vaccination. Indeed, strategies to ease restrictions on human mobility and interaction without provoking a major surge in transmission and mortality will depend on an accurate estimation of SARS-CoV-2 seroprevalence, which can be reliably achieved by massive serological testing. Serology has become indispensable not only for understanding the development and duration of immunity following infection or vaccination, but also for investigating the correlates of protection. Indeed, understanding the duration of protection and risk of reinfection after a natural infection or vaccination is crucial for planning the COVID-19 vaccination, especially with the emergence of more transmissible variants. However, most serologic assays, such as ELISA or Lateral Flow Immunochromatographic assays, either query the recognition of one antigen at a time or several antigens in bulk and provide limited information (IgG or IgM) on the quality of the anti-viral humoral response. Moreover, most of these tests have shown a wide range of performance, with many tests exhibiting inadequate sensitivity and specificity [3]. Yet, the need for accurate, extensive, and broad serological testing is required to understand the correlates of protection and to inform public health decisions.

Methods such as FCMBA immunoassays, which typically use beads with different fluorescent intensities coupled each to a specific antigen, have been used to simultaneously measure multiple viral antigen-specific antibody responses

[1] Department of Microbiology and Microbial Biotechnology Institute of Biology, Faculty of Natural Sciences and Mathematics «Sts Cyril and Methodius» University, 1000 Skopje, North Macedonia

[2] Department of Biochemistry and Molecular Pharmacology, NYU Langone Health, NY 10016, USA

[3] Department of Surgery, University of Miami, FL 33124, USA

in Human Immunodeficiency Virus [4,5]. This method outperforms ELISA for the detection of Herpes Simplex Virus type-specific immunoglobulins in terms of the number of antigens assayed, sample volume needed, and assay speed [6].

The concept and methodology of a flow bead array for comprehensive evaluation of humoral immune response and its depth post-viral infection and vaccination

FCMBA technology is a highly sensitive immunoassay whereby different groups of polystyrene carboxylated beads are impregnated with specific fluorescent dyes at varying intensities with the ultimate purpose of exploiting the ability of flow cytometry to detect the dynamic range of these fluorophores. The increasing fluorophore dye intensities should be optimized to enable discrimination of each bead group upon detection by flow cytometry at the specific wavelength of said fluorescent dye. In some examples, up to 11 discriminated bead groups can be identified per fluorescent dye based on intensity (Figure 1A). Each bead group is subsequently coupled with a specific viral antigen against which the humoral immune response will be assessed (Figure 1B). All bead groups are then pooled and added to the serum samples in a single reaction, enabling the antibodies in the serum sample to bind to their corresponding antigens that had been previously coupled to the beads (Figure 2A). Following incubation and wash, a mixture of detection antibodies labeled with different fluorescent dyes and targeting human isotypes such as IgG, IgM or IgA is added to the sample (Figure 2B). Henceforth, the sample can be acquired utilizing a flow cytometry platform (Figure 3A), and specifically bound detection antibodies can be individually distinguished by their respective wavelength channel (Figure 3B). Two discrimination channels can be used to evaluate flow cytometry data to identify different sets of antigen-coupled beads based on their intensity, (Figure 4A) and to distinguish

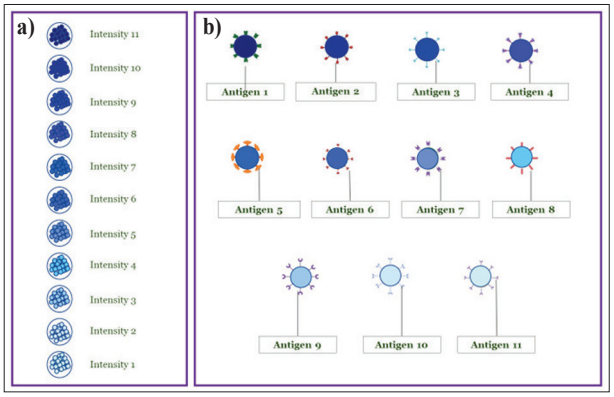

Figure 1. Definition of carboxyl particles and their coupling to proteins, antibodies, peptides, oligonucleotides, etc. – (a) beads contain internal dyes of different intensities; (b) chemistry occurs where the primary amine group of the target molecule (a protein, antibody, or peptide) interacts with the microsphere's carboxyl groups either directly or indirectly to generate a covalent amide bond.

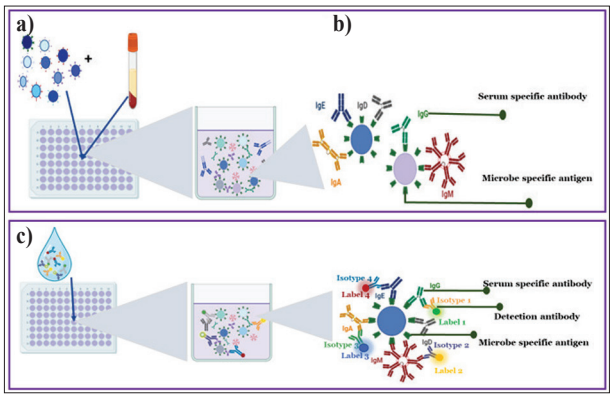

Figure 2. Bead array assay description – (a) distribution of the coupled beads with the targeted biomolecule (in this example, an antigen) in the 96-well microtiter plate, followed by the introduction of the experimental serum or plasma; (b) coupled beads bind and immobilize protein-specific antibodies; (c) antibodies that have been immobilized on the beads are differentiated and identified through the utilization of specific secondary antibodies that possess unique labels, regardless of whether they belong to the same or distinct isotypes.

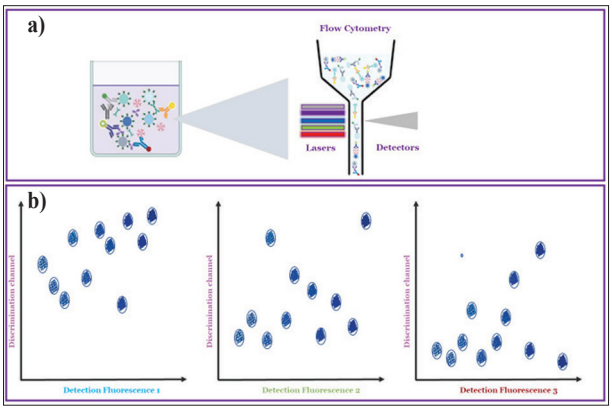

Figure 3. Executing the analysis – (a) acquisition of the samples using a flow cytometry platform; (b) producing and gathering data and aggregating the outcomes obtained from the various detection antibodies.

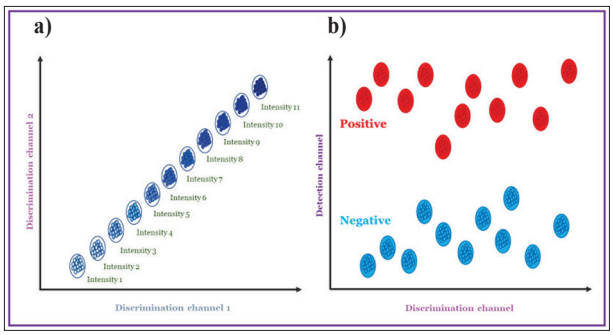

Figure 4. Data analysis obtained from flow cytometry using specialized software – (a) identifying individual sets of beads based on their intensity using two discrimination channels; (b) identification and differentiation of samples that test positive versus negative.

between samples that test positively versus negatively (Figure 4B). This assay can not only quantify multiple specific antibodies bound to their respective antigens but also determine the isotype of these antibodies, thereby allowing for the mapping of the humoral response and the determination of seroconversion status. The versatility of the assay could allow for the detection of large numbers of antibodies as more technical aspects of the FCMBA are taken advantage of, such as using various intensity beads of different dyes coupled to multiple antigens, and using the numerous available channels and specifications of the coupled flow cytometry platform.

Multiplexing the ability of FCMBA and COVID-19 serology evaluation

During pandemics, the detection of antibody responses using serological tests would allow for the monitoring of disease dissemination and vaccination responses. Highly modulable and flexible serological assays are needed to allow the inclusion of several antigens representing diseases as soon as they manifest. Implementations of FCMBA extended to the global COVID-19 pandemic, with its uses ranging from potential novel screening methods to the determination of disease severity [7,8]. Serological tests based on FCMBA technology are powerful techniques capable of detecting anti-SARS-CoV antibodies at a high throughput scale using only a few microliters of blood samples. Indeed, FCMBA technology was recently used to assess the serological profiles of SARS-CoV-2-naturally-infected or vaccinated individuals reacting to 11 antigens from human coronaviruses by measuring the total IgG, total IgA, and total IgM, as well as the IgA and IgG subtypes [9]. The serological profiling showed increasing levels of antibodies in the plasma of individuals after vaccination, including antibodies specific for the SARS-CoV-2 spike protein, which is targeted by the COVID-19 vaccines. Importantly, the assay also showed some cross-reactivity with the SARS-spike protein. FCMBA has also helped provide insight into the impact of the novel COVID-19 mRNA vaccinations on the immune systems, as it has been used by Takano et al. on vaccine recipients to identify variations in cytokine expression; differences in cytokine and chemokine expression were found to define six unique vaccine-induced immune dynamics in these patients [10]. COVID studies further used the cytokine/chemokine FCMBA as a supplementary means of defining factors associated with the severity of infection [11].

Furthermore, FCMBA technology allows for the testing of cross-neutralizing antibodies, which are antibodies that prevent a pathogen from entering cells, thus preventing the pathogen from replicating and protecting the organism from infection [12]. Detection of such antibodies can aid in passive antibody therapeutics as well as potential active prophylaxis. FCMBA can also be used for investigating the presence of cross-neutralizing antibodies against SAS-CoV-2 in dromedary camels [13]. Using a 5-antigen bead array, including SARS-CoV-2 proteins (S trimer, Membrane, Nucleoprotein, Envelope), and MERS-S protein, and sera of dromedaries that are MERS seropositive but MERS-CoV-free, the authors found that the tested dromedaries had medium-to-high titers of anti-MERS-CoV camel antibodies with variable cross-reactivity patterns against SARS-CoV-2 proteins. The discovery of SARS-CoV-2 cross-neutralizing camel antibodies in nonimmunized camels represents a promising venue for the development of SARS-CoV-2 hyperimmune camels, which could be a prominent source of therapeutic agents for the prevention and treatment of COVID-19.

Testing using FCMBA plays a crucial role in the convalescent plasma treatment, where plasma obtained from individuals who have recuperated from severe infections like COVID-19 is utilized. By conducting tests, it becomes possible to identify the specific plasma that can be employed for treatment based on its possession of highly effective neutralizing antibodies [14].

Further Preclinical and Clinical Usages

FCMBA technology allows for the timely measurement of various kinds of analytes, including cytokines, chemokines, antibodies, immunoglobulin isotypes, intracellular signaling molecules, and even single nucleotide polymorphisms (SNPs) [15]. The detection and analysis of these analytes have allowed for FCMBA to have broad medical applications on the bench-side and the bedside.

FCMBA has been used to aid in screening for disease in a robust and efficient manner with a small sample size. This has been mainly applied in autoimmune diseases, as was shown in the ability of a multiplex assay to comparatively detect antibodies against autoantigens in patients' sera [16]. Furthermore, FCMBA's use in disease screening also extended to allergies, with Zhou et al. observing anti-Poly Ethylene Glycol IgE antibodies developing in response to (PEG) ingestion [17]. FCMBA potential has also been noted in the early detection of some neurodegenerative diseases, as was shown in preclinical data regarding Alzheimer's disease [18]. The efficiency and practicality provided by FCMBA has also opened the door to allow for screening methods for diseases such as Herpes, Viral Hepatitis, Swine Fever and others in underserved areas [19,20]. The potential for FCMBA applications in diseases is not only limited to screening but also extends to helping identify crucial signatures involved in disease processes, as was shown by Bauer et al., revealing specific inflammatory differences between antibody associated demyelinating

diseases and multiple sclerosis [21]. Also revealing cytokine expression levels, Bai et al. have used FCMBA to compare differences in cytokine levels between patients with active tuberculosis, latent tuberculosis, and healthy patients, demonstrating a potential auxiliary efficacy to cytokines as biomarkers in the workup for tuberculosis [22].

CONCLUSION

Despite being a novel and exciting technology, the FCMBA system does have some disadvantages when compared to its ELISA counterpart. By virtue of being a more recent approach, the standardization and validation of diagnostic algorithms for flow-bead arrays are still not on par with the pre-established ELISA data; however, this will be overcome as more array kits are being approved for clinical and research use moving forward [23-25]. Furthermore, inherent to the assay involving multiple analytes and beads, there is a chance for cross-reactivity as the number of analytes increases, limiting the overall possible combinations of antibodies and ligands in the flow-bead microarray method [26,27].

That being said, FCMBA technology also offers multiple advantages. While ELISA-based arrays have the antibodies immobilized to a planar surface, FCMBA -based arrays could enable the binding of ligands to beads in suspension, allowing for a larger reaction area leading to faster binding kinetics [28]. The intrinsic multiplex nature of the FCMBA arrays allows for smaller sample sizes, which is advantageous when sample volume is limited, as is common in animal models and clinical studies. Furthermore, the smoother technical aspect of the suspension FCMBA method allows for faster and more cost-effective analysis when compared to ELISA-based methods [29]. This improved efficiency facilitates the application of FCMBA technology in personalized medicine, be it in allergy testing, autoimmune disease antibody detection, or the assessment of immune response [28]. From a technical standpoint, the suspension FCMBA immunoassay technology overcomes issues faced by the planar ELISA methods, such as mass-transportation limitations and printing and washing artefacts [30]. Through the use of FCMBA technology, the FCMBA arrays allow for enhanced automation in the analysis of the results, enhancing efficiency and reproducibility as compared to ELISA-based methods [31].

Overall, FCMBA provide a faster, more efficient method of detecting various analytes from a small sample, building on the base provided by the ELISA technique while also overcoming many of the limitations that exist in planar surface methods. This novel technique will pave the way for a more personalized approach to medicine, and will aid in screening, passive immune therapy and active prophylaxis against the current and any potential future pandemics.

REFERENCES

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Feb 20;382(8):727–33.
2. Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med. 2020 Jun;26(6):845–8.
3. Whitman JD, Hiatt J, Mowery CT, Shy BR, Yu R, Yamamoto TN, et al. Evaluation of SARS-CoV-2 serology assays reveals a range of test performance. Nat Biotechnol. 2020 Oct;38(10):1174–83.
4. Brown EP, Licht AF, Dugast AS, Choi I, Bailey-Kellogg C, Alter G, et al. High-throughput, multiplexed IgG subclassing of antigen-specific antibodies from clinical samples. J Immunol Methods. 2012 Dec 14;386(1–2):117–23.
5. Merbah M, Onkar S, Grivel JC, Vanpouille C, Biancotto A, Bonar L, et al. Standardization of a cytometric p24-capture bead-assay for the detection of main HIV-1 subtypes. J Virol Methods. 2016 Apr;230:45–52.
6. Binnicker MJ, Jespersen DJ, Harring JA. Evaluation of Three Multiplex Flow Immunoassays Compared to an Enzyme Immunoassay for the Detection and Differentiation of IgG Class Antibodies to Herpes Simplex Virus Types 1 and 2. Clin Vaccine Immunol CVI. 2010 Feb;17(2):253–7.
7. Egia-Mendikute L, Bosch A, Prieto-Fernández E, Lee SY, Jiménez-Lasheras B, García Del Río A, et al. Sensitive detection of SARS-CoV-2 seroconversion by flow cytometry reveals the presence of nucleoprotein-reactive antibodies in unexposed individuals. Commun Biol. 2021 Apr 20;4(1):486.
8. Zattoni IF, Huergo LF, Gerhardt ECM, Nardin JM, Dos Santos AMF, Rego FGM, et al. Multiplexed flow cytometric approach for detection of anti-SARS-CoV-2 IgG, IgM and IgA using beads covalently coupled to the nucleocapsid protein. Lett Appl Microbiol. 2022 Jun;74(6):863–72.
9. Rinchai D, Deola S, Zoppoli G, Kabeer BSA, Taleb S, Pavlovski I, et al. High-temporal resolution profiling reveals distinct immune trajectories following the first and second doses of COVID-19 mRNA vaccines. Sci Adv. 2022 Nov 11;8(45):eabp9961.

10. Takano T, Morikawa M, Adachi Y, Kabasawa K, Sax N, Moriyama S, et al. Distinct immune cell dynamics correlate with the immunogenicity and reactogenicity of SARS-CoV-2 mRNA vaccine. Cell Rep Med. 2022 May 17;3(5):100631.
11. Kwon JS, Kim JY, Kim MC, Park SY, Kim BN, Bae S, et al. Factors of Severity in Patients with COVID-19: Cytokine/Chemokine Concentrations, Viral Load, and Antibody Responses. Am J Trop Med Hyg. 2020 Dec;103(6):2412–8.
12. Li T, Xue W, Zheng Q, Song S, Yang C, Xiong H, et al. Cross-neutralizing antibodies bind a SARS-CoV-2 cryptic site and resist circulating variants. Nat Commun. 2021 Sep 27;12(1):5652.
13. Chouchane L, Grivel JC, Farag EABA, Pavlovski I, Maacha S, Sathappan A, et al. Dromedary camels as a natural source of neutralizing nanobodies against SARS-CoV-2. JCI Insight. 2021 Mar 8;6(5):e145785, 145785.
14. Kapil K, Muntode Gharde P. A Review on Effectiveness of Plasma Therapy in Severe COVID-19 Patients. Cureus. 14(9):e28914.
15. Morgan E, Varro R, Sepulveda H, Ember JA, Apgar J, Wilson J, et al. Cytometric bead array: a multiplexed assay platform with applications in various areas of biology. Clin Immunol Orlando Fla. 2004 Mar;110(3):252–66.
16. Chauhan R, Gupta N, Tiwari AK, Raina V, Nandi SP. Development of a Novel Multiplex Bead-based Assay for Measuring Autoantibodies on Flow Cytometric Platform. Immunol Invest. 2022 Apr;51(3):588–601.
17. Zhou ZH, Stone CA, Jakubovic B, Phillips EJ, Sussman G, Park J, et al. Anti-PEG IgE in anaphylaxis associated with polyethylene glycol. J Allergy Clin Immunol Pract. 2021 Apr;9(4):1731-1733.e3.
18. Del Prete E, Beatino MF, Campese N, Giampietri L, Siciliano G, Ceravolo R, et al. Fluid Candidate Biomarkers for Alzheimer's Disease: A Precision Medicine Approach. J Pers Med. 2020 Nov 11;10(4):221.
19. Yufenyuy EL, Vedapuri S, Zheng A, Cooley G, Danavall D, Mayur S, et al. Development of a Bead-Based Multiplex Assay for Use in Multianalyte Screening and Surveillance of HIV, Viral Hepatitis, Syphilis, and Herpes. J Clin Microbiol. 2022 May 18;60(5):e0234821.
20. Aira C, Ruiz T, Dixon L, Blome S, Rueda P, Sastre P. Bead-Based Multiplex Assay for the Simultaneous Detection of Antibodies to African Swine Fever Virus and Classical Swine Fever Virus. Front Vet Sci. 2019;6:306.
21. Bauer A, Rudzki D, Berek K, Dinoto A, Lechner C, Wendel EM, et al. Increased peripheral inflammatory responses in myelin oligodendrocyte glycoprotein associated disease and aquaporin-4 antibody positive neuromyelitis optica spectrum disorder. Front Immunol. 2022;13:1037812.
22. Bai R, Tao L, Li B, Liu A, Dai X, Ji Z, et al. Using cytometric bead arrays to detect cytokines in the serum of patients with different types of pulmonary tuberculosis. Int J Immunopathol Pharmacol. 2019;33:2058738419845176.
23. Coluccio ML, Presta I, Greco M, Gervasi R, La Torre D, Renne M, et al. Microenvironment Molecular Profile Combining Glycation Adducts and Cytokines Patterns on Secretome of Short-term Blood-derived Cultures during Tumour Progression. Int J Mol Sci. 2020 Jul 1;21(13):4711.
24. Garcia-Gerique L, García M, Garrido-Garcia A, Gómez-González S, Torrebadell M, Prada E, et al. MIF/CXCR4 signaling axis contributes to survival, invasion, and drug resistance of metastatic neuroblastoma cells in the bone marrow microenvironment. BMC Cancer. 2022 Jun 17;22(1):669.
25. Mattioda V, Benedetti V, Tessarolo C, Oberto F, Favole A, Gallo M, et al. Pro-Inflammatory and Cytotoxic Effects of Polystyrene Microplastics on Human and Murine Intestinal Cell Lines. Biomolecules. 2023 Jan 10;13(1):140.
26. Ellington AA, Kullo IJ, Bailey KR, Klee GG. Antibody-based protein multiplex platforms: technical and operational challenges. Clin Chem. 2010 Feb;56(2):186–93.
27. Nolan JP, Mandy F. Multiplexed and microparticle-based analyses: quantitative tools for the large-scale analysis of biological systems. Cytom Part J Int Soc Anal Cytol. 2006 May;69(5):318–25.
28. Elshal MF, McCoy JP. Multiplex bead array assays: performance evaluation and comparison of sensitivity to ELISA. Methods San Diego Calif. 2006 Apr;38(4):317–23.
29. Leng Y, Sun K, Chen X, Li W. Suspension arrays based on nanoparticle-encoded microspheres for high-throughput multiplexed detection. Chem Soc Rev. 2015 Aug 7;44(15):5552–95.
30. Nolan JP, Sklar LA. Suspension array technology: evolution of the flat-array paradigm. Trends Biotechnol. 2002 Jan;20(1):9–12.
31. Houser B. Bio-Rad's Bio-Plex® suspension array system, xMAP technology overview. Arch Physiol Biochem. 2012 Oct;118(4):192–6.