FLOW CYTOMETRY

MODULE DESCRIPTION/OVERVIEW

The module describes the main principle, parts and overview of flow cytometry. It describes the main applications of flow cytometry including both detection of immunomodulation accompanying disorders with high prevalence in Egypt, research, environmental and pharmaceutical applications.

The module represents an outline approach to the use and applications of flow cytometric immunophenotyping in the diagnostic immune-modulation laboratory. It shows how this technique could be used to study blood, bone marrow and tissue fluid samples in a variety of immune related scenarios to achieve a diagnosis and research, taking into account important features from the clinical history and alongside hematology, morphology, biochemistry, immunology, cytogenetic, histopathology and molecular data. The analysis of blood, bone marrow and tissue fluid specimens require a multi-faceted approach with the integration of scientific data from several disciplines. No single discipline can operate in isolation or errors will occur. Flow cytometry technology is in a privileged position in that it can provide rapid analysis of specimens; it is often the first definitive investigation to produce results and help formulate a working diagnosis.

This module highlights the expanding contribution of flow cytometry to basic biological research and diagnostic medicine. The utility of multiparametric flow cytometry is best demonstrated in identification of cells using cell tracking dyes, phenotypic markers, and viability probes and their function-based studies.

The complex progression of apoptotic death can be evaluated by monitoring multiple apoptotic characteristics simultaneously. Employing multiple antibodies to detect epitopes on cell cycle-regulated proteins provides more information than the measurement of DNA content alone.

Direct investigation of distinct cellular subsets in normal hematopoietic development versus hematologic diseases is critical to the understanding of disease initiation and progression. High-resolution polychromatic fractionation of hematopoietic precursors dissects developmental stages and identifies cellular subsets with defined lineage potentials. Commonly used protocols in the study of human hematologic disorders enable the diagnosis of patients with leukemia or primary immunodeficiency diseases. Cell-derived microparticles, which are implicated in pathogenesis, can be analyzed by conventional flow cytometry.

MODULE LEARNING OBJECTIVES

1) Providing the postgraduates with advanced knowledge, understanding, and critical judgment in Flowcytometry principles and development of protocols.
2) Enhancing the professional ability in diagnosis of immune disorders.
3) Enhancing the professional ability in research.

MODULE INTENDED LEARNING OUTCOMES

Upon successful completion of this module, students will be able to:

A- KNOWLEDGE AND UNDERSTANDING: (REMEMBERING AND UNDERSTANDING)

A1- Identify the different components of the instrument (Remember)

A2- Identify the ontogeny and the differentiation markers of different components of the peripheral blood and the bone marrow (Remember)

A3- List different fluorochromes (Remember)

A4- Explain the principle of fluorochromes operation and light compensation (understand)

A5- Demonstrate different sample processing

A6-Demonstrate different gating strategies for different cells (understand)

A7- Define abnormal cell populations encountered in different samples (Remember)

A8- Examine markers of immunomodulation, environmental toxicity and drug efficacy by flow cytometry. (Understand)

A9-Understand mechanism of cell sorting by flow cytometry (Understand)

B- INTELLECTUAL SKILLS: (APPLICATION, ANALYSIS, SYNTHESIS, EVALUATION)

B1- Apply staining protocols (Apply)

B2- Interpret flow cytometry data (Apply)

B3- Apply quality control measures in flow cytometry (Apply)

B4- Design flow cytometry experiment protocols (creation)

B5- Apply flow cytometry in detection of immune disorders related to immunodeficiency disease, cancer, chronic infection, sepsis, viral disease, and allergy (Apply)

B6- Apply safe lab practice during handling infectious samples (Apply)

B7- Apply flow cytometry in studying DNA analysis, Cell cycle, ploidy, apoptosis assay, and Cell viability (Apply)

B8- Apply flow cytometry in studying microparticles (Apply)

B9- Analyze pharmacokinetic by flow cytometry **(Analyze)**

B10- Asses the drug procedure activity and safety by flow cytometry. (Evaluate)

C- PROFESSIONAL SKILLS: (PRACTICAL SKILLS)

C1- Perform diagnostic and research flowcytometry tests eefficiently using different available techniques to identify the immune related disorders of different etiology.

C2- Perform instrument Set up with Quality Assurance procedures measures in different flowcytometric applications to control the total testing process

C3- Report laboratory findings in a complete informative formula.

C4- Perform sample processing with safe lab practice to maintain safe laboratory environment.

C5- Practice the complex data analysis

D- GENERAL SKILLS: (ATTITUDES AND COMMUNICATION SKILLS)

D1- Show self confidence in manipulating the flow cytometry protocols and data analysis.

D2- Respect the ethical consideration.

D3- Value effective communication with others in lab community.

D4- Posse work enthusiasm as appropriate for successful leadership.

D5- Value working cooperatively in a team group.

MODULE RESOURCES

Required Module Textbooks and Materials

1. Cabral-Marques O, Schimke LF, de Oliveira EB Jr, El Khawanky N, Ramos RN, Al-Ramadi BK, Segundo GRS, Ochs HD and Condino-Neto A (2019) Flow Cytometry Contributions for the Diagnosis and Immunopathological Characterization of Primary Immunodeficiency Diseases With Immune Dysregulation. *Front. Immunol.* 10:2742. doi: 10.3389/fimmu.2019.02742
2. Cossarizza A, Chang H-D, Radbruch A, Akdis M, Andrä I, Annunziato F, et al. Guidelines for the use of flow cytometry and cell sorting in immunological studies. Eur J Immunol. (2017) 47:1584–797. doi: 10.1002/eji.201646632
3. Crowley LC, Marfell BJ, Scott AP, Waterhouse NJ. Quantitation of Apoptosis and Necrosis by Annexin V Binding, Propidium Iodide Uptake, and Flow Cytometry. Cold Spring Harb Protoc. 2016 Nov 1;2016(11).
4. de Weck AL, Sanz ML, Gamboa PM, Aberer W, Bienvenu J, Blanca M, Demoly P, Ebo DG, Mayorga L, Monneret G, Sainte-Laudy J. Diagnostic tests based on human basophils: more potentials and perspectives than pitfalls. Int Arch Allergy Immunol. 2008;146(3):177-89. doi: 10.1159/000115885.

5. A flow cytometric method for characterization of circulating cell-derived microparticles in plasma. J Extracell Vesicles. 2014; 3: 10.3402/jev.v3.20795.
6. Ding M, Clark R, Bardelle C, Backmark A, Norris T, Williams W, Wigglesworth M, Howes R. Application of High-Throughput Flow Cytometry in Early Drug Discovery: An AstraZeneca Perspective. SLAS Discov. 2018 Aug;23(7):719-731. doi: 10.1177/2472555218775074. Epub 2018 May 22. PMID: 29787326.
7. Ebo DG, Bridts CH, Hagendorens MM, Aerts NE, De Clerck LS, Stevens WJ. Basophil activation test by flow cytometry: present and future applications in allergology. Cytometry B Clin Cytom. 2008 Jul;74(4):201-10. doi: 10.1002/cyto.b.20419. PMID: 18412216.
8. Elbim C, Lizard G. Flow cytometric investigation of neutrophil oxidative burst and apoptosis in physiological and pathological situations. Cytometry A. 2009 Jun;75(6):475-81. doi: 10.1002/cyto.a.20726. PMID: 19358285.
9. Elliott GS. Moving pictures: imaging flow cytometry for drug development. Comb Chem High Throughput Screen. 2009;12(9):849-859. doi:10.2174/138620709789383204
10. Hug S, Bernhard S, Stratmann AEP, Erber M, Wohlgemuth L, Knapp CL, Bauer JM, Vidoni L, Fauler M, Föhr KJ, Radermacher P, Hoffmann A, Huber-Lang M, Messerer DAC. Activation of Neutrophil Granulocytes by Platelet-Activating Factor Is Impaired During Experimental Sepsis. Front Immunol. 2021 Mar 16;12:642867. doi: 10.3389/fimmu.2021.642867. PMID: 33796110; PMCID: PMC8007865.
11. Joslin J, Gilligan J, Anderson P, et al. A Fully Automated High-Throughput Flow Cytometry Screening System Enabling Phenotypic Drug Discovery. SLAS Discov. 2018;23(7):697-707. doi:10.1177/2472555218773086
12. Kalina T. Reproducibility of flow cytometry through standardization: opportunities and challenges. *Cytometry A.* (2019). doi: 10.1002/cyto.a.23901.
13. Mebius, R.E.: Organogenesis of lymphoid tissues. *Nat. Rev. Immunol.* 2003,3:292–303.
14. Melamed, M. R. (2001) A brief history of flow cytometry and sorting. Methods in Cell Biology 63(part A), pp. 3–17.
15. Newbold A, Martin BP, Cullinane C, Bots M. Detection of apoptotic cells using propidium iodide staining. Cold Spring Harb Protoc. 2014 Nov 3;2014(11):1202-6.
16. Payne K, Li W, Salomon R, Ma CS. OMIP-063: 28-Color Flow Cytometry Panel for Broad Human Immunophenotyping. *Cytometry A* (2020) 97(8):777–81. doi: 10.1002/cyto.a.24018.
17. Shapiro, H. M. (2003) Practical Flow Cytometry, 4th editon. Wiley-Liss, New York
18. Takashima T, Okamura M, Yeh T, Okano T, Yamashita M, Tanaka K, et al. . Multicolor flow cytometry for the diagnosis of primary immunodeficiency diseases. J Clin Immunol. (2017) 37:486–95. 10.1007/s10875-017-0405-7
19. Virginia Litwin, Philip Marder. Flow Cytometry in Drug Discovery and Development. 2011; ISBN:978-0-470-43356-0

20. Watson, J. V. (2004) Introduction to Flow Cytometry. Cambridge University Press, Cambridge
21. Watson, J. V. (2005) Flow Cytometry Data Analysis: Basic Concepts and Statistics. Cambridge University Press, Cambridge.
22. Welinder, E., Ahsberg, J., and Sigvardsson, M.: B-lymphocyte commitment: identifying the point of no return. Semin. Immunol. 2011, 23:335–340.
23. Wintrobe's Clinical Hematology. Greer JP.,Foerster J, Rodgers GM, Paraskevas F, Glader B, ArberDA and Means RT. Editors.Wolters Kluwer/Lippincott-Williams&Wilkins comp. Philadelphia,Baltimor, NewYork, London.

-ASSIGNMENTS AND GRADING SCHEME

GRADING SYSTEM

Diagnostic: level assessment before the course

Formative: Exit cards, quiz, interaction during demonstrations given periodically during course, Portifolio and DOPs

Summative: at the end of the course duration.

- **Written theoretical multidimensional exams** with MCQ, SAQ, problem solving and True or False to assess student knowledge & understanding as well as intellectual abilities regarding the theory and practice of clinical microbiology.
- **Practical exam** to assess student intellectual abilities as well as professional and practical skills gained from the course e.g. **OSPE**

GRADING POLICY

Grades can be based on the following:

Practical presentations and assignments	20%
Exams	70%
Class attendance/participation	10%
Total Points	**100%**

MODULE POLICIES

LATE ASSIGNMENTS

.....