REPORT:

FLOW CYTOMETRY WORKSHOP

ORGANIZED BY

SGCP's
Guru Nanak Khalsa College
of Arts, Science & Commerce (Autonomous)

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INDIAN COUNCIL OF MEDICAL RESEARCH | NATIONAL INSTITUTE FOR RESEARCH IN REPRODUCTIVE AND CHILD HEALTH

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ACKNOWLEDGEMENT

I am grateful to Dr. Ratna Sharma, our principal of Guru Nanak Khalsa College of Arts, Science & Commerce, for granting permission to hold this workshop, which I had the honor of attending. In addition, I would like to sincerely thank the esteemed academic members of the Department of Bioinformatics and Guru Nanak Khalsa College, especially Dr. Surekha Gupta, Head of the Zoology Department, whose vision and leadership made this workshop possible. I also want to express my gratitude to the committed instructors in the zoology department for helping to put together this amazing course.

In addition, I want to thank the professors of our bioinformatics department – Dr. Gursimran Kaur Uppal, Ms. Aparna Patil Kose, and Ms. Sermarani Nadar—for their guidance and mentoring both before and during the workshop.

We also extend our sincere gratitude to the distinguished speakers from the Indian Council of Medical Research (ICMR), whose knowledge and perceptions have broadened our understanding. The knowledge of flow cytometry has been enhanced by the efforts of Dr. Vikrant M. Bhor, Dr. Maya Gupta, Dr. Vainav Patel, and other ICMR members. Your willingness and eagerness to share your expertise and insights has motivated us to investigate the seemingly endless opportunities in the industry.

This program has given me a wealth of information, insights, and skills that will definitely help me in my academic and professional endeavors. I would like to express our sincere appreciation to everyone who helped make this session a success once more. Your commitment has sparked an enthusiasm for study and research that will keep us striving for greatness in the bioinformatics discipline.

Figure 1: Department of Bioinformatics at the Flow Cytometry event

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CHAPTER 1 – INTRODUCTION OF THE EVENT

The Indian Council of Medical Research-National Institute for Research in Reproductive and Child Health (ICMR-NIRRCH), Mumbai, and the Department of Zoology, Guru Nanak Khalsa College of Arts, Science & Commerce (Autonomous), Matunga collaborated to organize the Flow Cytometry Workshop on September 21, 2024, in the banquet hall of the latter institution.

With the goal of providing participants with a thorough understanding of flow cytometry—a crucial tool in cell biology that enables the careful examination of cell populations based on their chemical and physical characteristics—as well as the operational mechanisms, elements, underlying ideas, and uses for flow cytometry. The event was sponsored by Tata Consultancy Services (TCS), highlighting the importance of industry funding to support medical research education initiatives.

Students, professors, and professionals excited about the applicability of flow cytometry across several biological study fields were among the large crowds drawn to the event. The program was thoughtfully designed by the organizers to combine academic knowledge with real-world application, guaranteeing that participants gained the essential practical skills for carrying out flow cytometry investigations.

The seminar was divided into four sessions led by experts in their respective fields. The first session, 'Flow Cytometry Basics', introduced the principles and techniques of flow cytometry by Dr. Vikrant Bhor. He clarified the importance of this method in modern biological research and made it accessible to attendees with varying levels of knowledge. The second session, 'Sample Preparation', emphasized the importance of proper sample preparation for accurate flow cytometry results by Dr. Maya Gupta. She provided guidance on best practices and common mistakes. The third session, 'Viral Immunopathogenesis', focused on using flow cytometry to study immune responses to viral infections. Dr. Vainav Patel discussed real-world examples to demonstrate the technique's significance in medical research. Finally, a hands-on and demonstration session allowed participants to lymphocyte subset analysis using a flow cytometer, reinforcing the theoretical knowledge gained earlier. This interactive session encouraged and facilitated learning from experienced instructors.

Schedule of the Workshop

Date	Day	Session	Speaker
21st September, 2024	Saturday	**Session 1:** Basics of Flow Cytometry	Dr. Vikrant Bhor
		Session 2: Sample Preparation for Flow Cytometry	Dr. Maya Gupta
		Session 3: Viral Immunopathogenesis	Dr. Vainav Patel
		Session 4: Demonstration of the Flow Cytometry	Dr. Maya Gupta

CHAPTER 2 – INTRODUCTION OF THE ESTEEMED SPEAKERS FOR THE DAY, THE ORGANIZERS AND THE SPONSORS

1. Dr. Vikrant Bhor (Speaker)

Dr. Vikrant Bhor is a distinguished Scientist E in the Department of Molecular Immunology and Microbiology at the ICMR-National Institute for Research in Reproductive and Child Health. His research focuses on critical areas such as host-pathogen interactions, microbial biofilms, and the microbiome-immune axis, with particular attention to congenital infections, reproductive tract infections (RTIs), sexually transmitted infections (STIs), and HIV.

Dr. Bhor has received notable recognition for his contributions to the field, including the Rapid Grant for Young Investigators from the Department of Biotechnology (DBT) in 2013 and a Post-doctoral Fellowship from the Curie Institute in 2006. His latest publications include significant studies on vaginal microbiota variations in women from a community clinic in Mumbai, India, and a novel gut microbiome-immune axis influencing pathology in HCMV-infected infants with neonatal cholestasis, both published in 2023.

2. Dr. Maya Gupta (Speaker)

Dr. Maya Gupta is a renowned immunologist and cytometry expert who has made significant contributions to the field of flow cytometry in biological and clinical research. Her expertise lies in evaluating complex cellular populations and their characteristics, such as size, complexity, and marker expression, to understand disease mechanisms and treatment effects.

Dr. Gupta's work has expanded the field of cytometry and provided crucial insights into the interactions between immune cells, cancers, and infections, leading to the development of innovative diagnostic and therapeutic strategies. She is actively involved in Cytometry India, an organization that promotes the advancement of cytometric methods and their applications, demonstrating her extensive knowledge and leadership in the field.

Throughout her career, Dr. Gupta has published extensively in prestigious journals, disseminating her research on cellular responses and immunological profiling in various disease states. Her efforts have significantly impacted the use of flow cytometry in both basic and clinical research, making her a prominent figure in the field. By leveraging this powerful technology, Dr. Gupta has elucidated crucial aspects of immune function and disease, contributing to a better understanding of these complex processes.

3. Dr. Vainav Patel (Speaker)

Dr. Vainav Patel is a prominent Scientist E in the Department of Viral Immunopathogenesis at the ICMR-National Institute for Research in Reproductive and Child Health. His research primarily focuses on viral pathogenesis and genomics, immunology, and vaccine design.

Dr. Patel's contributions to the field have been recognized through several prestigious awards, including the Global Scholar Award from the American Society for Virology (ASV) in 2023, an award for his contributions to COVID-19 efforts from the Metropolis Foundation in 2022, and the Keystone Symposia Global Health Travel Award by the Bill & Melinda Gates Foundation in 2020. His latest publications include significant studies on SARS-CoV-2 infection dynamics in both unvaccinated and vaccinated populations in Mumbai, as well as a retrospective analysis of SARS-CoV-2 positivity in neonates born to COVID-19 positive mothers, both published in 2023.

In addition to his research, Dr. Patel serves as a member of the Board of Studies for Life Sciences & Biochemistry at St. Xavier's College (Autonomous), Mumbai, and Molecular Biology at Mumbai University. He is also an adjunct faculty member at the Centre of Excellence in Immunology (CoE), ICMR-NIRRCH & BYL Nair Hospital, where he teaches immunology to medical students, and a visiting faculty member at the Centre of Excellence in Basic Sciences, University of Mumbai, teaching advanced immunology since 2013.

4. ICMR – NIRRCH (Organizer)

The ICMR-National Institute for Research in Reproductive and Child Health (NIRRCH) is a prominent research institution under the Indian Council of Medical Research (ICMR). Located in Mumbai, NIRRCH focuses on advancing knowledge and developing interventions related to reproductive health, maternal and child health, and adolescent health.

The institute conducts extensive research on various aspects of reproductive health, including infertility, sexually transmitted infections, and maternal complications. It also emphasizes the importance of child health by investigating neonatal and pediatric health issues. NIRRCH plays a crucial role in addressing public health challenges through its research initiatives, contributing to policy formulation and implementation in India.

In addition to its research activities, NIRRCH collaborates with national and international organizations to enhance its scientific capabilities and outreach. The institute aims to improve healthcare outcomes through evidence-based practices and innovative solutions in reproductive and child health.

CHAPTER 3 – SESSIONS

Session 1 – Basics of Flow Cytometry
Speaker – Dr. Vikrant Bhor

Flow Cytometry

Flow cytometry is an advanced analytical technique widely used in various fields, including immunology, molecular biology, and clinical diagnostics. It allows for the rapid multi-parametric analysis of single cells as they flow through a laser beam in a fluid stream.

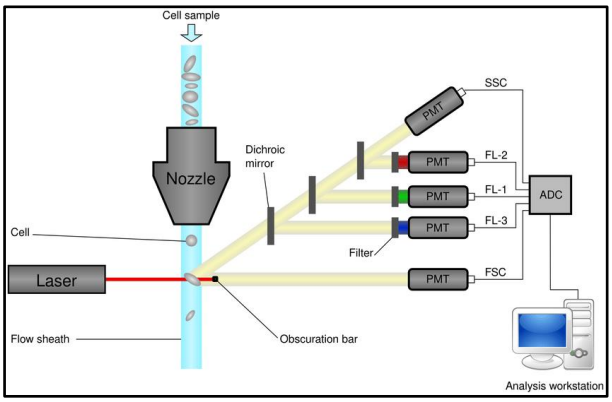

Figure 2: Flow Cytometer

Microscope vs. Flow Cytometry

Researchers examine cell populations using flow cytometry and microscopy, which gives them unique insights into the architecture and functions of the cells. The identification of individual cellular components is made possible by the thorough observation of cells and their architecture made possible by light microscopy in particular. Advanced kinds of microscopy, including fluorescence microscopy, employ fluorescent dyes to highlight components within cells, yielding higher-resolution insights.

Flow cytometry, on the other hand, objectively evaluates the physical and chemical features of cells as they flow via a laser beam, providing extensive information on cell size, granularity, and the presence of particular surface markers. This technology is particularly beneficial for immunological investigations, allowing researchers to study immune cell populations and their roles under varied situations, such as infections or malignancies.

Components of Flow Cytometer

1. **Laser System:** An essential part of Fluorescence Detection and Counting (FACS) is the laser system, which supplies the light source required to excite fluorescent dyes affixed to certain cell surface markers. Since different lasers produce light at different wavelengths, it is possible to detect numerous fluorescent markers at once. Typical laser wavelengths for FACS are 640 nm (red), 405 nm (violet), and 488 nm (blue). The selection of lasers improves the capacity of researchers to discriminate between different cell kinds and states by allowing them to assess a broad range of factors using various fluorescently tagged antibodies.

2. **Fluidics System:** The sample must be moved through the cytometer via the fluidics system. To concentrate the sample into a narrow stream, a sheath fluid—typically a buffered saline solution—is usually used. This makes sure that each cell or particle passes the laser beam separately, enabling precise measurement. A constant flow rate and pressure are maintained by the fluidics system's cooperating parts, which comprise pumps, tubing, and flow cells,

guaranteeing peak performance throughout analysis. In order to guarantee that the single cell makes it past the laser by hydrodynamic focusing of the sample, the fluidics system is necessary.

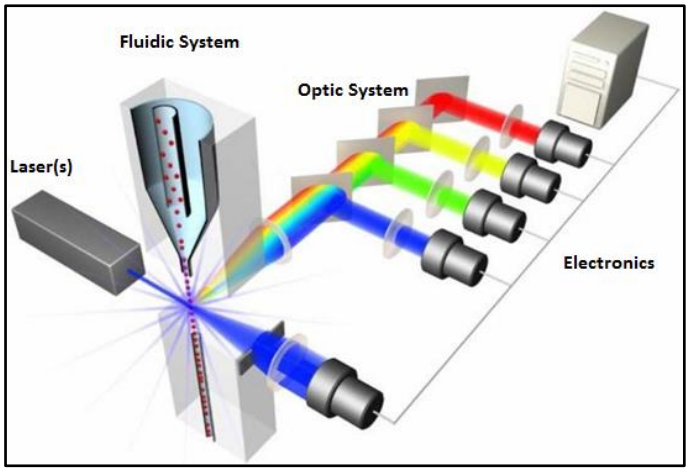

Figure 3: Components of a flow cytometer

3. **Optics System:** The optics component includes lasers, lenses, filters, and detectors. Lasers provide the excitation light needed to illuminate the fluorescently labeled cells. The emitted light is collected and filtered to isolate specific wavelengths corresponding to different fluorescent markers, allowing for detailed analysis of cell characteristics.

Type	Property	Description
Excitation Optics	Lasers	Emit light at specific wavelengths to excite fluorescent dyes on cells or particles.
	Positioning and Focusing	Lasers are precisely positioned and focused to illuminate the sample as it passes through the cytometer.
	Laser Coherence and Intensity	Essential for reliable fluorescence signals.
Collection Optics	Light Scattering and Fluorescence	Cells scatter light and emit fluorescence after interacting with the laser light.
	Collection of Emitted Light	Mirrors and lenses are used to collect this emitted light.
	Optical Filters	Separate different light wavelengths and direct specific signals to detectors.
	Detectors	Photomultiplier tubes (PMTs) or photodiodes are commonly used to detect the collected light signals.

4. Detectors

Detector Type	Description	Advantages	Disadvantages
Photomultiplier Tube (PMT)	Traditional detector with high sensitivity and low fluorescence detection capability. Converts light signals into electrical signals.	High sensitivity, low fluorescence detection	Bulky, high voltage requirements
Avalanche Photodiode (APD)	Solid-state detector with high sensitivity and linearity, especially for red fluorescence detection.	High sensitivity, linearity, solid-state	Higher noise than PMTs
Silicon Photodiode (SiPD)	Cost-effective solid-state detector for fluorescent signal detection.	Cost-effective, good performance	Lower sensitivity compared to PMTs and APDs
Imaging Detector (CCD/CMOS)	Captures images of cells for multi-parameter analysis at single-cell and population levels.	Provides detailed images, multi-parameter analysis	Higher data volume, slower acquisition
Spectral Detector	Measures the entire emission spectrum from each particle, enabling analysis of multiple fluorescent markers without spectral overlap.	Eliminates spectral overlap, enhances multi-parameter experiments	More complex data analysis

5. Visualization of Flow Cytometry Data

Key steps involved in data visualization and analysis for flow cytometry –

a. **Gating –** Use forward scatter (FSC) and side scatter (SSC) to identify cell populations based on size and granularity. Apply gates to exclude debris, dead cells, and doublets.

b. **Histograms –** Analyze single parameters to identify positive and negative populations for a specific marker. Overlay negative controls to accurately determine positive populations.

c. **Dot Plots –** Visualize two parameters simultaneously in a density plot. Identify distinct cell populations based on marker expression. Quantify relative proportions of different cell types.

d. **Compensation –** Account for spectral overlap between fluorochromes. Measure single-stained controls to generate a compensation matrix. Apply compensation to the data to correct for spillover.

e. **Automated Gating –** Use mathematical modeling to analyze high-dimensional data. Employ clustering algorithms like PCA, SPADE, and tSNE. Extract meaningful insights from complex datasets.

f. **Data Pre-processing –** Remove technical artifacts and low-quality data. Exclude doublets by plotting FSC-H vs FSC-A. Ensure proper compensation and gating before downstream analysis.

Fluorescence – Activated Cell Sorting (FACS)

A method used in flow cytometry called Fluorescence-Activated Cell Sorting (FACS) allows cells to be recognized and arranged according to their fluorescence properties. To identify different cell populations within a heterogeneous mixture, it binds to particular cell surface markers with antibodies that have been fluorescently tagged. Size, granularity, and protein expression are among the factors that are analyzed using the fluorescence and light scattering data that have been gathered.

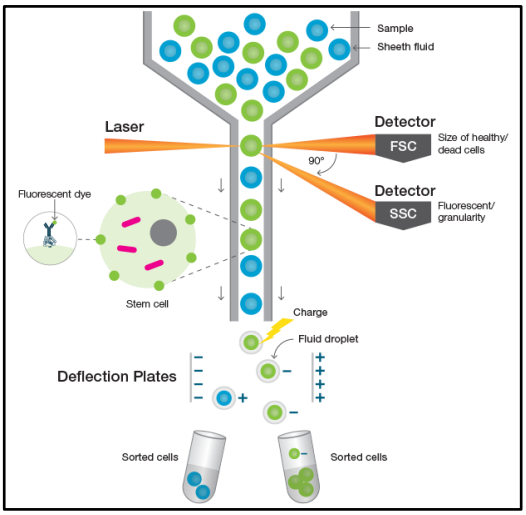

Figure 4: Workflow of FACS

Feature	Flow Cytometry	FACS
Primary Function	Analyzing cells without sorting	Analyzing and sorting cells
Cell Analysis	Measures cell size, granularity, and fluorescence	Measures cell size, granularity, and fluorescence
Sorting	Does not sort cells	Sorts cells based on fluorescence characteristics
Sample Fate	Typically discards samples after analysis	Collects sorted cells for further study
Applications	Immunology, cancer research, stem cell biology	Immunology, cancer research, stem cell biology
Key Difference	Focuses solely on analysis	Combines analysis with sorting

Applications of Flow Cytometry

1. **Cell sorting –** From a heterogeneous mixture, specialized flow cytometers may physically separate and collect particular cells of interest, allowing for further examination or cultivation of these cells.
2. **Detection of Apoptosis –** This method examines the morphological and biochemical alterations in dying cells to differentiate between necrosis and apoptosis, two forms of cell death.
3. **Immunophenotyping –** The most popular use of flow cytometry in identification and quantification of various cell types based on particular surface markers is immunophenotyping. When identifying haematological cancers like lymphoma and leukaemia, it is highly beneficial.

4. **Analysis of Cell Cycle –** Flow cytometry is a useful tool for understanding cell proliferation and identifying aneuploidy linked to chromosomal abnormalities. It can evaluate the many phases of the cell cycle.
5. **Assays for Cell Proliferation –** Flow cytometry assesses the metabolic activity of cells in response to external stimuli, which enables the evaluation of cell activation and proliferation.
6. **Measurement of Intracellular Calcium Flux –** This program keeps track of the quantities of calcium ions in cells, which are essential for a number of signaling pathways.
7. **Applications in Microbiology –** Flow cytometry measures microbial susceptibility to antibiotics and assists in the detection of bacterial and viral illnesses.
8. **Genetic analysis** is utilized in prenatal testing, carrier detection, and karyotyping.
9. **Pharmacology –** The method aids in the investigation of drug reactions and cell dynamics.

Session 2 – Sample Preparation for Flow Cytometry
Speaker – Dr. Maya Gupta

Immune System

The immune system is a sophisticated network of cells and proteins that defends the body against pathogens, including viruses. It consists of two main components:

1. **Innate Immunity:** The first line of defense, providing immediate but non-specific protection against infections.
2. **Adaptive Immunity:** A more specialized response that develops over time, involving T cells, B cells, and memory cells that provide long-lasting immunity.

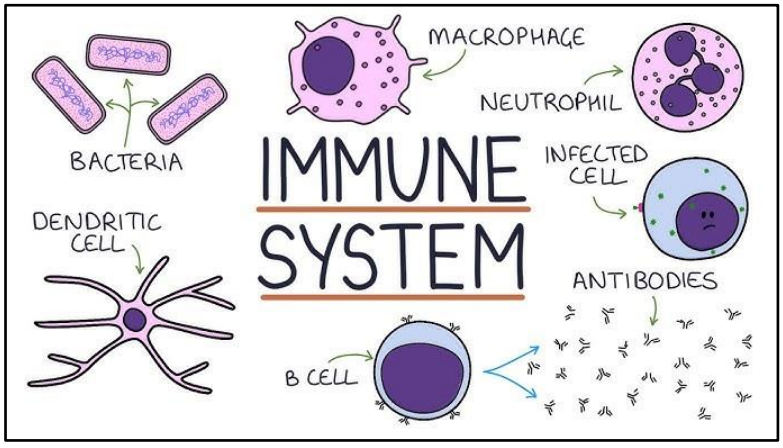

Figure 5: Immune system

T Cells

T cells play a pivotal role in the adaptive immune response and can be categorized into:

1. **CD4+ Helper T Cells:** These cells assist other immune cells by releasing cytokines and are crucial for orchestrating the immune response.
2. **CD8+ Cytotoxic T Cells:** They directly kill virus-infected cells by recognizing specific antigens presented by major histocompatibility complex (MHC) molecules.

Additionally, **memory T cells** are formed after an infection or vaccination, allowing for a faster and more robust response upon re-exposure to the same pathogen.

B Cells

B cells are responsible for producing antibodies that neutralize viruses. Their development occurs in two stages:

1. **Antigen-Independent Development:** Occurs in the bone marrow where B cells mature and express unique receptors.
2. **Antigen-Dependent Development:** When B cells encounter specific antigens, they proliferate and differentiate into plasma cells that produce antibodies or memory B cells that provide long-term immunity.

Natural Killer (NK) Cells

NK cells are part of the innate immune system and play a critical role in recognizing and destroying virus-infected cells without prior sensitization. They are characterized by their ability to identify stressed or infected cells through various receptors.

Lymphocyte Subset Analysis

The evaluation of lymphocyte subsets (T cells, B cells, NK cells) is essential for understanding immune responses during viral infections. Flow cytometry is a powerful tool used to enumerate these subsets based on specific surface markers:

1. **T Cells:** Identified using CD3 (pan-T cell marker), CD4 (helper), and CD8 (cytotoxic).
2. **B Cells:** Identified using CD19.
3. **NK Cells:** Identified using CD16 and CD56.

This analysis helps in diagnosing immunodeficiencies, monitoring infections, and evaluating responses to therapies.

Flow Cytometry Techniques

Flow cytometry is instrumental in viral immunopathogenesis research due to its ability to analyze individual cells rapidly. Several techniques enhance its utility:

1. **Use of Primary and Secondary Antibodies**
   In flow cytometry, primary antibodies bind specifically to target antigens on cell surfaces or within cells. Secondary antibodies, which are typically conjugated with fluorescent dyes, bind to primary antibodies to amplify the signal for detection. This dual-antibody approach enhances specificity and sensitivity in detecting viral proteins or immune cell markers.

2. **Multiplexed Assays**
   Multiplex assays allow simultaneous detection of multiple targets within a single sample using different fluorescently labeled antibodies. This capability is crucial for comprehensive profiling of immune responses during viral infections.

3. **Fluorescence Minus One (FMO) Controls**
   FMO controls are used to determine the background fluorescence level when analyzing flow cytometry data. By excluding one fluorochrome at a time from the panel, researchers can accurately set gates for positive populations without misclassifying negative events.

4. **Antibody Titration**
   Determining optimal antibody concentrations is essential for minimizing background noise while maximizing signal detection. Titration helps establish the best conditions for staining samples before flow cytometric analysis.

Sample Handling and Preparation

1. **Source of Sample and Transport**
   Samples for flow cytometry can be obtained from various sources, including blood or tissue biopsies. Proper transport conditions (e.g., temperature control) are critical to preserving cell viability and function until analysis.

2. **Cell Isolation**
   Isolating specific cell populations from mixed samples can be achieved using density gradient centrifugation or magnetic bead separation techniques. This step ensures that only the desired lymphocyte subsets are analyzed.

3. **Sample Preparation Techniques**
   Flow cytometry sample preparation can follow different protocols:
   a. **Single Platform:** Involves direct staining of whole blood or isolated cells before analysis.
   b. **Double Platform:** Involves initial isolation followed by staining.

Sample Preparation Protocols	Steps	Notes
Lyse-No Wash (LNW)	1. Add lysis buffer to whole blood. 2. Incubate. 3. Stain. 4. Analyze.	Simplifies sample prep, ideal for lymphocyte analysis in whole blood.
Tube Lysing or Stain-Lyse-Wash (SLW)	1. Stain cells. 2. Lyse. 3. Wash. 4. Analyze.	Suitable for isolated cells or whole blood, allows for removal of unbound antibodies.
Bulk Lysing or Lyse-Stain-Wash (LSW)	1. Lyse. 2. Wash. 3. Stain. 4. Wash. 5. Analyze.	Effective for larger sample volumes, ensures bulk lysis before staining.

Steps in Sample Preparation

STEP 1: Erythrocyte Lysis

- **Purpose:** To remove red blood cells (RBCs) from whole blood, enriching the lymphocyte population for analysis.

- **Procedure:**
  1. Prepare an erythrocyte lysis buffer (e.g., ammonium chloride buffer). A common formulation (10X stock) includes:

Ammonium chloride	82.6 g
EDTA	0.37 g
Potassium phosphate	10 g
Distilled water	1L

  2. Mix 2 mL of whole blood with 18 mL of 1X erythrocyte lysis buffer.
  3. Invert the tube gently 10 times to mix.
  4. Incubate at room temperature (20-25°C) for 2 minutes.
  5. Centrifuge at 400 x g for 5 minutes to pellet the white blood cells (WBCs).
  6. Carefully remove the supernatant to discard lysed RBCs, leaving a pellet of WBCs.

↓

STEP 2: Staining Tests

- **Purpose:** To label specific cell populations with fluorescently labeled antibodies.
- **Procedure:**
  1. Resuspend the WBC pellet in an appropriate buffer (e.g., PBS) at a concentration of approximately 1-10 million cells/mL.
  2. Add specific fluorescently labelled antibodies targeting cell surface markers (e.g., CD3, CD4, CD8, CD19).
  3. Incubate in the dark at 4°C for 30 minutes to 1 hour to allow binding.
  4. Ensure gentle mixing during incubation to maintain cell viability.

↓

STEP 3: Fixation and Permeabilization

- **Purpose:** To preserve cellular structures and allow access to intracellular targets.
- **Procedure:**
  1. After staining, wash cells with PBS to remove unbound antibodies by centrifuging at 400 x g for 5 minutes and discarding the supernatant.
  2. Fix cells using paraformaldehyde (typically at a final concentration of 1-4%) or another fixative for 10-20 minutes at room temperature.
  3. If analyzing intracellular proteins, permeabilize cells using a permeabilization buffer (e.g., saponin or Triton X-100) as per protocol requirements.

↓

STEP 4: Washing

- **Purpose:** To remove excess unbound antibodies and reduce background fluorescence.
- **Procedure:**
  1. Wash cells with PBS or flow cytometry buffer (e.g., FACS buffer containing BSA) by centrifuging at 400 x g for 5 minutes.
  2. Repeat this step 2-3 times to ensure thorough washing.

↓

STEP 5: Storage

- **Purpose:** To preserve samples until analysis can be performed.
- **Procedure:**
  1. Resuspend cells in TransFix solution or paraformaldehyde in PBS if immediate analysis is not possible.
  2. Store samples at 4°C for short-term storage (up to several days) or freeze at -80°C for long-term storage, depending on the specific requirements of the experiment.

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STEP 6: Acquisition

- **Purpose:** To run samples through a flow cytometer for data acquisition.
- **Procedure:**
  1. Ensure that the flow cytometer is calibrated and properly set up with appropriate laser settings and compensation controls before running samples.
  2. Acquire data from each sample, ensuring that enough events are collected for statistical validity (typically at least 10,000 events).

↓

STEP 7: Gating and Dot Plot Analysis

- **Purpose:** To analyze data and identify specific cell populations based on fluorescence intensity and scatter properties.
- **Procedure:**
  1. Use software tools associated with the flow cytometer to create dot plots based on forward scatter (FSC) vs. side scatter (SSC) to distinguish live cells from debris and dead cells.
  2. Apply gating strategies to isolate populations of interest based on fluorescent markers (e.g., T cells vs. B cells).
  3. Analyze results for specific markers, comparing populations across different conditions or treatments.

Troubleshooting and Compensation

During flow cytometry experiments, issues such as spectral overlap between fluorochromes may arise, necessitating compensation adjustments to ensure accurate data interpretation. Troubleshooting involves –

1. Checking instrument settings.
2. Verifying antibody specificity.
3. Ensuring proper sample preparation protocols were followed.
4. Ensuring that all reagents are prepared fresh and kept on ice when necessary to maintain cellular integrity.
5. Using sterile techniques throughout to prevent contamination.
6. Adjusting centrifugation speeds based on specific protocols or cell types being analyzed.

Session 3 – Viral Immunopathogenesis
Speaker – Dr. Vainav Patel

Viral immunopathogenesis is a critical field of study that focuses on understanding how viruses interact with the host immune system and the resulting pathological effects. It encompasses the processes by which viruses enter the body, replicate, spread, and ultimately cause tissue damage and disease. Understanding these mechanisms is crucial for developing effective treatments and preventive measures against viral infections.

Key Concepts in Viral Immunopathogenesis

1. **Virus Entry and Replication –** Viruses typically enter the host through mucosal surfaces or breaks in the skin. Once inside, they must infect susceptible cells to replicate. The site of entry often dictates the subsequent pathogenesis; for instance, respiratory viruses primarily target lung epithelial cells, leading to pneumonia or acute respiratory distress syndrome (ARDS) in severe cases.

2. **Immune Response –** The host's immune response plays a dual role in viral infections. While an appropriate immune response can eliminate the virus, an exaggerated or dysregulated response can lead to immunopathology, resulting in tissue damage and severe disease outcomes. For example, in COVID-19, excessive cytokine production (cytokine storm) can cause significant lung injury and multi-organ failure.

3. **Pathological Mechanisms –** Viral pathogenesis involves both direct damage caused by viral replication within cells and indirect damage mediated by the immune response. Some viruses induce cell death directly through lysis or apoptosis, while others may trigger inflammatory responses that inadvertently harm surrounding tissues.

4. **Clinical Outcomes –** The clinical manifestations of viral infections can range from asymptomatic to severe disease, influenced by factors such as viral load, host genetics, pre-existing immunity, and co-infections. For instance, emerging viruses like Nipah virus and Monkeypox can cause severe outcomes due to their ability to evade immune responses.

This discipline encompasses various viral diseases, including COVID-19, Monkeypox, and the Nipah virus, each presenting unique challenges for public health and requiring tailored research approaches.

COVID-19

COVID-19, caused by the SARS-CoV-2 virus, has highlighted the importance of viral immunopathogenesis. The virus employs various mechanisms to evade the immune response, leading to severe disease in some individuals.
Research in this area has focused on understanding the dynamics of viral replication, immune evasion strategies, and the development of effective vaccines.

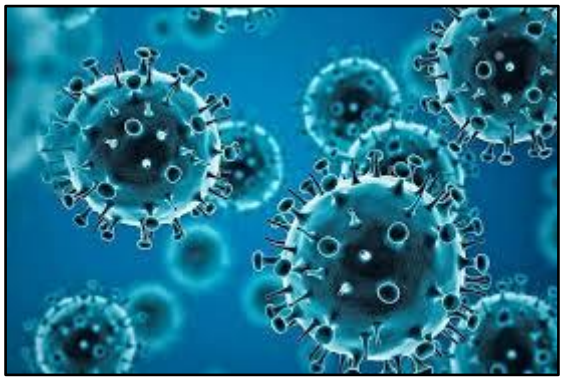

Figure 6: COVID-19

Studies have shown that flow cytometry plays a vital role in characterizing immune responses to COVID-19, helping researchers evaluate vaccine efficacy and monitor immune cell populations in infected individuals.

Monkeypox

Monkeypox is another viral disease that has gained attention due to recent outbreaks. It is caused by the monkeypox virus, which is closely related to smallpox. Understanding the immunopathogenesis of monkeypox involves studying how the virus triggers immune responses and causes disease manifestations.
Research efforts are geared towards developing vaccines and therapeutics, with flow cytometry facilitating the analysis of immune cell responses during infection.

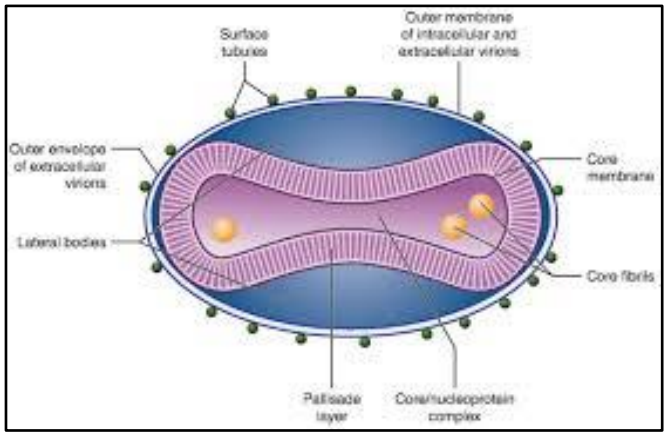

Figure 7: Structure of Monkeypox virus

Nipah Virus

The Nipah virus is a zoonotic pathogen associated with high mortality rates. It poses significant public health risks, especially in regions where it is endemic. Research into Nipah virus immunopathogenesis focuses on understanding its transmission dynamics and immune evasion mechanisms. Flow cytometry aids in investigating the host immune response to Nipah virus infection, providing insights into potential vaccine targets and therapeutic strategies.

Figure 8: Nipah virus

Role of Flow Cytometry in Viral Immunopathogenesis

1. **Single-Particle Analysis –** Flow cytometry allows for the detection and characterization of individual viral particles through a technique known as flow virometry. This capability enables researchers to analyze viral populations and their properties rapidly, which is crucial for understanding viral behavior and interactions with host cells.

2. **Immunophenotyping –** It provides detailed information about immune cell populations and their functional states. By assessing various markers on immune cells, researchers can determine how these cells respond to viral infections and vaccines.

3. **High Throughput –** Flow cytometers can analyze thousands of cells per second, allowing for robust statistical analyses that enhance our understanding of viral infections across diverse populations. This rapid data generation is vital for monitoring outbreaks and evaluating public health interventions.

4. **Versatility –** The technology can be adapted to measure a wide range of biological components, including proteins, RNA, and DNA from both viruses and host cells. This versatility makes flow cytometry invaluable in studying complex interactions within the host-pathogen dynamic.

In summary, viral immunopathogenesis is pivotal in addressing emerging viral threats like COVID-19, Monkeypox, and Nipah virus. Flow cytometry serves as a cornerstone technology in this field, enabling precise analysis of viral particles and host immune responses, ultimately contributing to better diagnostics, therapeutics, and vaccine development strategies.

Session 4 – Demonstration of the Flow Cytometry
Speaker – Dr. Maya Gupta

The lymphocyte subset analysis method was used to illustrate how a flow cytometer operates. Analyzing lymphocyte subsets involves identifying and measuring various lymphocyte subtypes in a sample, usually blood. The immune system's main building block is the lymphocyte, which comes in several forms such T cells (CD4+ and CD8+), B cells, and natural killer (NK) cells. Understanding immune function, identifying immunological diseases, tracking the course of disease, and assessing treatment responses all depend on the analysis of these subsets. Flow cytometry is used for the analysis, enabling the simultaneous measurement of several characteristics on a single cell.

Lymphocyte Subset Analysis

1. **Sample Preparation –** Blood samples are typically collected in anticoagulant tubes. Red blood cells are lysed to enrich the lymphocyte population, which can be done using protocols such as Lyse-No Wash (LNW), Stain-Lyse-Wash (SLW), or Lyse-Stain-Wash (LSW).

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2. **Staining –** Cells are stained with fluorescently labeled antibodies that target specific surface markers. Common markers include:
    a. CD3: Pan-T cell marker.
    b. CD4: Helper T cells.
    c. CD8: Cytotoxic T cells.
    d. CD19: B cells.
    e. CD56: NK cells.

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3. **Flow Cytometry Analysis –** The stained cells are passed through a flow cytometer, where they are illuminated by lasers. The emitted fluorescence is detected and analyzed based on intensity and wavelength, allowing for the identification of different cell populations.

Figure 9: Beckman Coulter Flow Cytometer

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4. **Gating Strategies –** Gates are applied to exclude debris and dead cells while isolating specific lymphocyte populations based on their forward scatter (FSC) and side scatter (SSC) characteristics. This process helps in accurately quantifying the subsets of interest.

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5. **Data Interpretation –** The results are typically presented in histogram or dot plot formats, showing the distribution of lymphocyte subsets. For example, a histogram may display the percentage of CD4+ T cells within the total lymphocyte population.

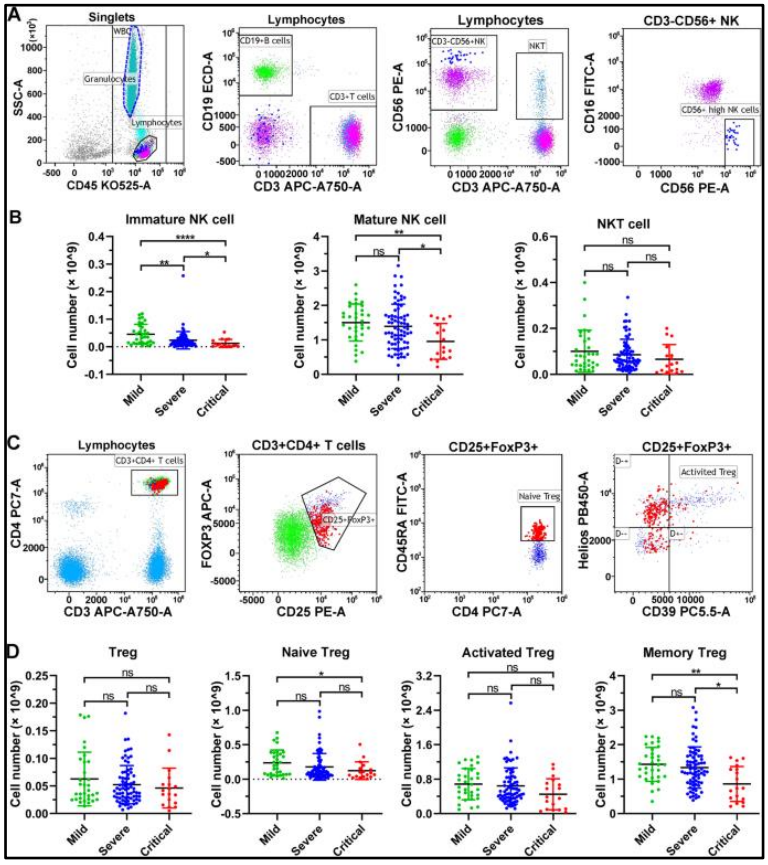

Figure 10: Characteristics of lymphocyte subset alterations in COVID-19 patients with different levels of disease severity

Applications

1. **Clinical Diagnostics –** Lymphocyte subset analysis is crucial for diagnosing immune disorders, monitoring HIV progression, and evaluating responses to therapies.
2. **Research –** It aids in understanding immune responses in various conditions, including infections, autoimmune diseases, and cancers.

CHAPTER 4 – KEY TAKEAWAYS

1. **Fundamental Principles –** Participants gained a solid understanding of the basic principles of flow cytometry, including how it measures multiple characteristics of individual cells in a fluid stream.
2. **Fluorophore and Panel Design –** The workshop covered the selection and design of appropriate fluorophores for multi-color experiments, essential for effective immunophenotyping.
3. **Data Acquisition and Analysis –** Attendees learned how to perform data acquisition using flow cytometers, as well as techniques for data analysis using software.
4. **Compensation Techniques –** The importance of compensation for spectral overlap between different fluorescent markers was emphasized, along with methods to perform it accurately.
5. **Statistical Analysis –** Participants were introduced to required controls and statistical methods necessary for reliable data publication.
6. **Applications in Research –** The workshop highlighted various applications of flow cytometry, including immunophenotyping, cell cycle analysis, apoptosis detection, and analysis of phosphoproteins and soluble proteins.
7. **Hands-On Experience (Demonstration) –** Practical sessions allowed participants to apply theoretical knowledge in real-world scenarios, enhancing their skills in designing experiments and analyzing results.
8. **Networking Opportunities –** The workshop provided a platform for participants to connect with experts and peers in the field, fostering collaboration and knowledge sharing.

CHAPTER 5 – REFERENCES

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