Association for the Advancement of Blood & Biotherapies

Bringing Flow Cytometry To Life Applications in Cellular Therapies

An APPROACH based on user experience with different flow applications and array of cell types.

Syllabus

Technical Requirements and Contact Information for Assistance

This course is offered entirely online as independent, self-paced study through AABB's Education Platform located at http://education.aabb.org.

Technical Requirements - learners must:

- Have an internet connection to access the course
- Navigate and be able to use the features of the course content and Education Platform

We strongly recommend that registered learners use either the Google Chrome, Safari, Microsoft Edge or Firefox browsers to access the course. Anyone using MAC or PC can download Google Chrome. Internet Explorer is not supported.

For questions related to the program including access submit an enquiry via email to the AABB eLearning team at eLearning@aabb.org. A response should be expected Monday – Friday during business hours (US Eastern Standard Time or EST) within 48 hours of request.

Course Description

Flow cytometry plays an integral role in the modern laboratory helping to elucidate cell phenotype, characterization, and function. The number of parameters and speed of simultaneous measurements in single cells or events has continued to increase with advances in hardware, reagents and analytical software. Learning more about how this tool is applied in actual practice is essential to maximizing its power and further drive (or support) clinical and research applications specific to cellular therapy (CT).

Through narrated presentation and interactive exercises, this course will bring flow cytometry to life focusing on its functional uses as it relates to cellular therapy. Real examples and protocols for sample preparation, data collection, gating strategies for an array of cell types, data analysis, common problems encountered and how this technology is applied will be demonstrated.

The course is presented in 3 sections highlighting important aspects of flow cytometry practice and application for CT:

- **Section 1: Foundational Concepts in Flow Cytometry** - describes the technology, what you should know, and how the technology can be optimized providing an overview of the scientific and technical concepts of basic flow cytometry setup.
- **Section 2: Cellular Therapy Applications Using Flow Cytometry** - presents examples of how the technology is used in a variety of applications commonly used in CT providing a variety of case studies demonstrating how flow cytometry concepts are applied in practice.
- **Section 3: Review of Concepts: Applying What You Have Learned** - brings the concepts together. You should be able to synthesize principles and applications brought together in practical interactive exercises as practical challenges in real-world flow.

Content Description and Learning Objectives

Following completion of this course you should be able to meet the learning objectives listed in each section:

Section 1: Foundational Concepts in Flow Cytometry

Description: Understanding of flow cytometry technology, function and processes involved in sample preparation and measurement is important to ensuring consistent and reliable results. This section describes the technology and the ways in which flow cytometry can be optimized for CT products.

Learning objectives:

- ✓ Describe the concept of targeting cell surface markers with antibody-fluorochrome conjugates.
- ✓ Identify and describe the essential technical components of a flow cytometry assay.
- ✓ List the major practical steps in assay setup and performance.
- ✓ Explain how event data are collected and analyzed.

Section 2: Cellular Therapy Applications of Flow Cytometry

Description: There are a variety of methods to apply flow cytometry to a range of different CT products. This section presents information on application of current flow cytometric methods to a variety of cell types serving both as current practices information as well as an introduction to methods that may prove useful in thinking about approaches to other flow strategies for future products.
This section presents examples of how flow cytometry is used in a variety of applications.

Learning Objectives:
Apply flow cytometry concepts to assays characterizing the following types of CT products:

- ✓ Match cell(s) of interest with corresponding CT product type.
- ✓ Discuss how to optimize a flow panel for staining cells based on CT product to be evaluated.
- ✓ Identify cell types based on flow cytometric characterization.
- ✓ Describe gating strategies used to distinguish and quantify cell populations of interested on flow cytometer dot plots.
- ✓ Enumerate cell populations of interest based on flow cytometric analysis.

The CT products characterized:

- Hematopoietic Progenitor Cell (HPC) product
  - HPC, Apheresis [HPC(A)]
  - HPC, Marrow [HPC(M)]
  - Donor Lymphocyte Infusion (DLI) from HPC product
- Mononuclear Cell (MNC) product
  - T Cell/Lymphocyte products using cell surface markers
    - Donor Lymphocyte Infusion (DLI)
    - Chimeric Antigen Receptor (CAR) T cell
    - Transgenic T Cell Receptor (TCR) T cell
    - Natural Killer Cell (NKC)
  - Virus-Specific T Cell (VST) using intracellular cytokine staining (ICS)
  - Monocyte-Derived Dendritic Cell (MoDC) Vaccine
- Mesenchymal Stromal Cells (MSC)

Section 3: Review of Concepts: Applying What You Have Learned

Description: Standardization of flow cytometry testing is very important for delivery of consistent quality CT products particularly with the increase in multi-center trials. With development of some cell types there may not be a "one size fits all" approach, but there are general points to consider in standardization. This section pulls the concepts together testing your knowledge and things to consider in a series of exercises providing feedback.

Learning objectives:

- ✓ Recognize cellular and flow cytometric characteristics that are critical to obtaining useful information on understanding the properties by completing exercises.
- ✓ Formulate an approach to designing flow cytometry panels to assess various cellular therapies.
- ✓ Describe a general approach to gating strategies.

Course Content Accessibility

The course content is narrated. The volume level can be adjusted by moving the volume icon (visible on the screen), in the preferred direction and/or adjusting your own computer volume controls.

When you 'click' on each course section, the first slide, which is not narrated, will automatically present the name of the course and the faculty responsible for creating the content.

Each section is divided into different topic headings representing a slide set. You will be able to advance the slide set or return to those slides you wish to review by selecting the "next" or "previous" command button. You can also select the slide from the list in the side panel. We recommend that you first view the material in its intended order for best understanding. You will always be able to return to slides for review as many times as you wish during your access period.

Key ideas, bulleted lists, images, and illustrations support the narrative. Interactive exercises provide feedback to see how well you have captured information as you work through. Written transcripts and glossaries that explain the terms are located via tabs in the side panel.

Additional references, journal articles, and web sources are included for further information. To view these, click on the "References" link that appears in each section.

Activities for Successful Completion of the Course

Read and study all materials for each section, including additional readings that support and reinforce basic information presented. Take time to answer questions that ask you to reflect on the material. Follow the sections in order as information builds upon preceding lessons. Since this is a self-paced program, learners may decide how much time is needed to review and study the materials. It is estimated the course will take ~ 4-6 hours to complete. A suggested strategy is to create a study plan or timeline for completing each section. Follow that plan to ensure timely completion within the year that you will have access to the course.

Self-Assessment

A self-assessment is provided after viewing all three sections of the course. It is an additional study tool to gauge your level of comprehension of the content presented in the course. Passing the self-assessment is not required in order to earn continuing education credits and/or complete the course. The self-assessment is a pass/fail exercise where learners will have 4 opportunities to achieve 80% or higher. There is no grade provided for the self-assessment; rather an opportunity to reinforce your understanding of the content provided in the program. On the self-assessment:

- Learners will receive questions (i.e., multiple choice, true-false questions, matching) and asked to select the correct answer.
- Learners will receive feedback as to whether an answer choice is correct or incorrect.
- Feedback with the correct answers will be provided with the 3rd attempt.

Continuing Education Credits

This course is eligible for 6 continuing education credits/contact hours for General Participation, California Lab Personnel or Florida Lab Personnel. The number and type of credits awarded for this course was determined by the estimated program completion time. This course is not eligible for continuing education credit for physicians or California Nurses. For more information on each credit type please visit our Continuing Education Credits webpage. A continuing education certificate of completion will be immediately provided to learners upon reviewing all 3 sections of the course and completion of the course evaluation.

Conferral of Continuing Education Credits

For successful completion of the course resulting in conferral of continuing education credits:

1. Read and carefully study each slide in all sections. You will not be able to advance to each section of the course without completing the prior section.
2. Complete the AABB "Bringing Flow Cytometry to Life" course evaluation.

Learners will automatically receive via the AABB Education Platform a continuing education certificate that includes continuing education credits when learners have completed all sections of the course, and the Course Evaluation. Learners will also be able to access the continuing education certificate in the My Transcripts section of the AABB Education Platform.

Course Access

Access to the course in the AABB Education Platform will be available to you for 1 year from the date of registration for the course (immediate access is granted upon registration). This course is self-paced; however, you must complete all sections of the course and a course evaluation within the year to receive the Continuing Education Certificate. If you are unable to complete the course within the one-year period and still wish to complete the course, then you must re-purchase the course. Questions related to registration should be directed to eLearning@aabb.org.

Course Faculty & Contributors

Faculty

Andrew Fesnak, MD, Director, Cell Manufacturing & Development, Clinical Cell & Vaccine Production Facility, University of Pennsylvania, Philadelphia, PA

Dr. Fesnak is a transfusion medicine physician and cell therapy researcher at the University of Pennsylvania Perelman School of Medicine. He is the director of Cell Manufacturing and Development in the Clinical Cell and Vaccine Production Facility. He shares medical oversight of the Apheresis Clinic and leads a manufacturing group dedicated to developing next generation cell therapies.

Steven Highfill, PhD Research and Product Development Supervisor, Center for Cellular Engineering (CCE) at National Institutes of Health (NIH), Bethesda, MD

Dr. Highfill leads Chemistry, Manufacturing, and Controls (CMC) activities for new cell therapy products — building and managing project plans to achieve clinical manufacturing goals that support NIH Clinical Center Investigational New Drugs (INDs). Collaborating with Clinical Center investigators, clinical research managers, and regulatory agencies, Dr. Highfill assures that processes and assays are developed in compliance with FDA regulations to produce consistent biological products. The CCE is located within the Clinical Center of NIH and currently supports >50 active INDs from investigators within, or in affiliation with, the NIH Clinical Center. The clinical trials are predominately Phase I/II, and often first-in-human studies. Products currently in the clinical manufacturing process include T-cells (CART, TCR, virus-specific), mesenchymal stem cells, dendritic cells, natural killer cells, monocytes, and genetically modified hematopoietic stem cells (HSCs).

Emily Hopewell, PhD, **MT,** Director of Cell & Immunotherapy Manufacturing, Indiana University School of Medicine, Indianapolis, IN

Dr. Hopewell has more than a decade of cellular therapy and laboratory experience. She received a BA in Microbiology and a BS in Clinical Laboratory Science from Miami University in 2002 and 2003, respectively, and obtained her medical technologist certification in 2003. She completed her PhD in cancer biology at the University of South Florida in 2012. Her career in cell therapy began in 2003 at the Moffitt Cancer Center as a technologist processing and analyzing cells for both standard of care and experimental cellular therapies. After completing her PhD, she returned to the Moffitt Cancer Center Cell Therapy team as a development specialist, then as assistant director. She is currently the director of cell and gene therapy manufacturing and an assistant clinical professor at Indiana University and is charged with implementing GMP cell manufacturing at Indiana University. She has extensive experience with flow cytometry for both standard of care hematopoietic stem cell therapy, as well as the next generation of cell and gene therapy.

Jo Lynn Procter, MEd, MT, SBB (ASCP), CQA (ASQ), Center for Cellular Engineering, Department of Transfusion Medicine, NIH Clinical Center, National Institutes of Health, Bethesda, MD

Jo Procter earned her BS in microbiology with a medical technology option from Pennsylvania State University in 1979. She worked as a medical technologist for 40 years. She studied blood bank technology at Michael Reese Research Foundation in Chicago, IL, in 1982 and received a Master of Education degree with a concentration in health services from Augusta College in Augusta, GA, in 1989. She began work in the department of transfusion medicine at NIH in 1991 as technical specialist, Immunohematology Reference Laboratory, transitioned to the Cell Processing Section's Flow Cytometry Laboratory in 2001, and became supervisor of operations for what is now the Center for Cellular Engineering in 2004. Proctor retired from NIH in May 2019.

Suzanne Thibodeaux, MD, PhD, Assistant Professor, Washington University in St. Louis School of Medicine, Saint Louis, MO

Dr. Thibodeaux earned her MD and PhD degrees at the University of Texas Health San Antonio. As part of her graduate work, which involved investigation of tumor immune therapies, she utilized flow cytometry to evaluate immune cell phenotypes and function. She completed a residency in clinical pathology and a fellowship in blood banking and transfusion medicine at the Hospital of the University of Pennsylvania. Dr. Thibodeaux then joined the Department of Pathology and Immunology at Washington University School of Medicine in St. Louis, MO. She is currently medical director of the Cellular Therapy Laboratory and assistant medical director of transfusion services at Barnes-Jewish Hospital. Her clinical and research interests focus on improving cellular therapies from the clinical laboratory perspective, as well as apheresis and transfusion medicine as it relates to cellular therapy.

Contributors

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