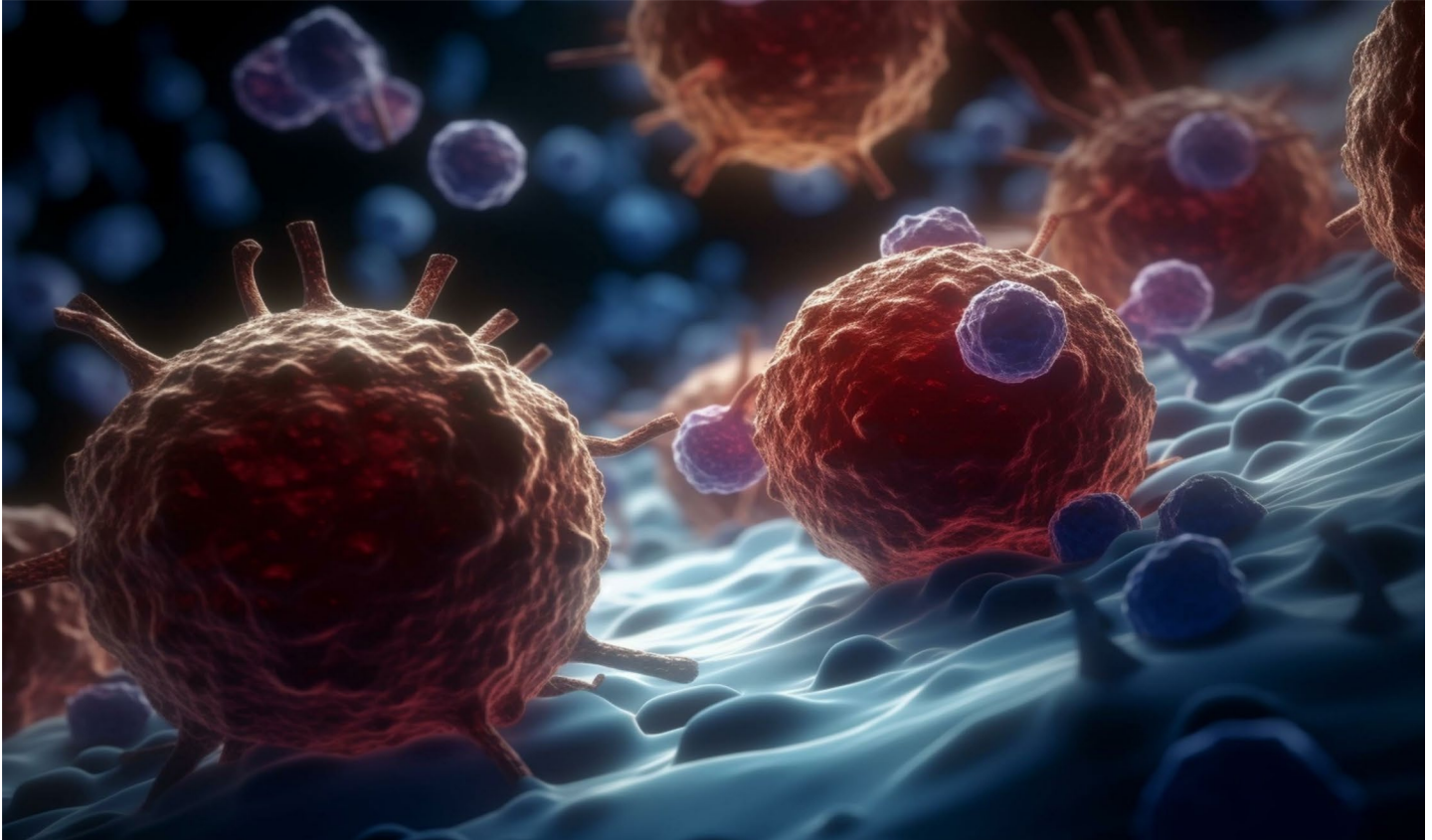


Face to Face starts the 3rd week of class in Biology B412

LABORATORY OF IMMUNOLOGY



MICR 4154

THE UNIVERSITY OF TEXAS AT EL PASO

Dr. Anna M. Eiring PhD and Dr. Jen Apodaca PhD

BIOLOGICAL RESEARCH SCIENCES

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SYLLABUS FOR MICR 4154 FALL 2025

INTRODUCTION TO IMMUNOLOGY LABORATORY (MICR 4154 FALL 2025)

COURSE INFORMATION

LAB DIRECTOR: Dr. Anna Eiring

LAB COORDINATOR: Dr. Jennifer Apodaca

LABS AND TEACHING ASSISTANTS:

Day	Start Time	End Time	CRN	Teaching Assistant	email
Wednesday	9:00 AM	11:50 AM	11425	Aylin Sanchez	Asanchez110@miners.utep.edu
Wednesday	1:30 PM	4:20 PM	11353	Aylin Sanchez	Asanchez110@miners.utep.edu
Thursday	10:30 AM	1:20 PM	11354	Stephanie Gutierrez	skgutierrez@miners.utep.edu
Thursday	1:30 PM	4:20 PM	14686	Stephanie Gutierrez	skgutierrez@miners.utep.edu

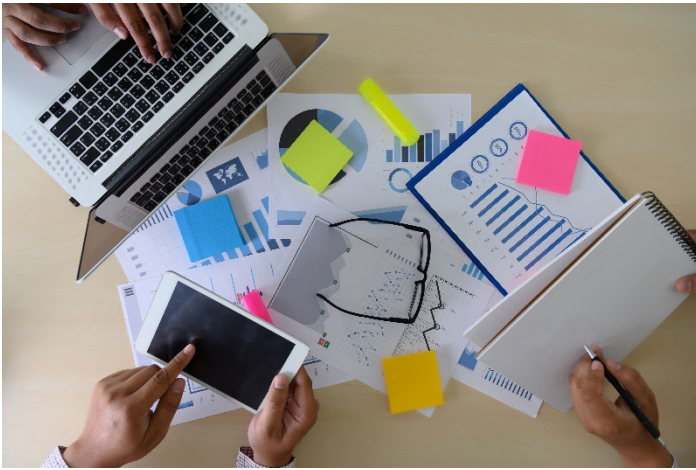
OFFICE HOURS: The Instructor of Record and Teaching Assistants will post office hours and accommodate all students as time permits. Students may request an appointment with the coordinator in person or via email. The course coordinator will try to respond as soon as possible (generally within 24-48 hours during the school week). When sending an email, send through the Blackboard® course email or place the course number (CRN) and name (e.g., MICR4154 11605 and the issue/topic in the subject line of the email). All appointments should be made at least 2 business days in advance. Dr. Apodaca will also provide virtual office hours that are synchronous through a UTEP-approved platform (MS Teams, Blackboard, or Zoom) and these appointments are best used to ask about research proposals, and scientific literature related to your project.

REQUIRED RESOURCES

The following materials and resources that you will frequently use in this lab:

- BLACKBOARD - USED TO DOWNLOAD MATERIALS AND UPLOAD ASSIGNMENTS
- LAB MANUAL
- LAB COAT
- SAFETY GLASSES (SEE BLACKBOARD)
- LAB NOTEBOOK
- LAPTOP/TABLET WHEN APPROPRIATE
 - Students are expected to bring laptop computers to the class for participation in exercises or assessments as indicated by the course syllabus. It is the responsibility of the students to make sure that the laptops are in working condition.
 - In a major disruption (e.g., H1N1 epidemic, subzero weather), be prepared to maintain course progress via other means (e.g., Internet, our Blackboard course shell, etc.) and check your email (especially your UTEP miners account) regularly. Make sure you have checked the following:
 - Audio (speaker & microphone) and video (camera) MUST be checked to be functional for classes
 - Students should be ready at any time to share their screen, camera, audio with classmates/faculty for online course learning situations
- MICROSOFT® TEAMS OR UTEP ZOOM®, MS TEAMS MAY BE USED IN LAB TO COMPLETE ASSIGNMENTS.

OVERVIEW AND LAB STRUCTURE



We will convene once a week for a three-hour session. Due to the nature of working with cells and the experimental procedures, there may be occasional in-lab follow-ups. The laboratory exercises will center on acquiring specific skills and techniques. These will encompass the analysis of primary literature, qualitative and quantitative research methods, microscopy, flow cytometry, ELISA, and more. It's important to recognize that these skills are pivotal not only for crafting your group research proposal but also for successfully completing the laboratory projects.

During this period, you will formulate a research proposal that you will write throughout the semester. The laboratory manual and activities contain background information on

immunology and potential research questions focusing on cytokine expression in neutrophils and macrophages. Subsequently, working in groups, you will select a research question and draft a research proposal.

The graduate student Teaching Assistant (TA) and the instructor of record will act as research mentors, providing guidance to each group. Your TA will also check your lab notebook several times during the semester and assign a grade. Importantly, while the TA is primarily responsible for facilitating the lab, we are committed to ensuring that you have a successful and enjoyable experience and will be visiting the lab routinely to facilitate project development and implementation.

COURSE EXPECTATIONS AND LEARNING OUTCOMES:

*What's the difference between a learning **goal** vs. a learning **objective**?*

Learning goals refer to the higher-order ambitions, while learning objectives are the specific, measurable competencies which we assess in order to decide whether your goals have been met.

By the end of this course students should demonstrate the following skills, knowledge, and competencies:

LAB LEARNING GOALS

This laboratory course and the accompanying lecture course provide an integrated approach to studying an immunological question using a variety of techniques in the field of immunology.

Our objectives in the laboratory are:

- To develop familiarity and understanding of standard immunological procedures and instrumentation.
- To understand the appropriate laboratory techniques used for cell culture.
- To learn the safe handling of cell cultures, reagents, and equipment in the laboratory.

LAB LEARNING OBJECTIVES

- Apply fundamental concepts in immunology to propose a research experiment.
- Develop critical thinking and problem-solving skills and extrapolate information from scientific literature and experimentation.
- Communicate your understanding of immunology and what you have learned from your experiments.

TYPICAL LAB SCHEDULE

- Before lab: Read and Review the "Background", "Objectives", "Activities" and supplemental materials on Blackboard before entering the lab.
- Lab start: Place bags and all other non-essential items in the cabinet. Food and drinks must be placed outside.
- In-lab discussion and/or demonstration of lab procedures (10-15 minutes).
- Prepare the work area and determine roles for the experiment. Approximately 90 minutes to complete the lab.
- Exit Quiz: Part of your exit quiz includes cleaning your work area and the lab.

- The last 30 minutes of the lab is reserved for clean-up and waste disposal according to protocol and biosafety procedures.

TEAMWORK AND ROLES

During this semester, students will collaborate in groups to design and write a research proposal. However, within each group, every student will assign themselves a specific role with a defined list of tasks associated with that role. This approach allows students to work effectively within a group dynamic while also establishing individual responsibilities, much like the setup in a research laboratory. Consequently, your grade for this laboratory course will be composed of both group-based and individual components. The roles for this semester are outlined in today's in-class activity below, and each group will have the autonomy to decide which student undertakes each role. For details of the specific requirements for teamwork, please review "Laboratory 1"; we will work on this during the first day of lab.

IMMUNOLOGY LABORATORY (MICR 4154) COURSE POLICIES LAB RULES AND EXPECTATIONS:

GENERAL STATEMENT ABOUT COURSE POLICIES:

The Individual Course and Common language syllabi are subject to change to meet course needs, especially if there are unexpected disruptions or changes in class size, resources, etc. The most updated individual course syllabus can be found on the course Blackboard shell. It is the student's responsibility to review the syllabus periodically for updates. If syllabus language is unclear, students need to seek clarification with the course coordinator.

ATTENDANCE: Attendance in the course is determined by participation in the learning and lab activities of the course. Your participation in the lab is important not only for your learning and success but also to create a community of learners. Regular attendance is expected. You are assigned to a specific lab section; attending another section is not permitted. If you're unable to attend due to reasons such as a conference, UTEP sports obligations, school interviews, or COVID symptoms, notify your TA and instructor of record with proper documentation. Arrangements will be made accordingly. Participation is determined by completion of the following activities:

- Reading/Viewing all course materials to ensure understanding of assignment requirements.
- Participating in engaging discussion in lab, and working through experiments, (grading rubric provided in the "grading information" area of each forum).
- Other activities as indicated by the lab syllabus.

EXCUSED ABSENCES AND/OR COURSE DROP POLICY: According to UTEP Catalog, "At the discretion of the instructor, a student can be dropped from a course because of excessive absences or lack of effort. A grade of "W" will be assigned before the course drop deadline and a grade of "F" after the course drop Deadline. See Policies and Regulations in the UTEP Undergraduate Catalog for a list of excused absences. Therefore, if I find that, due to non-performance in the course, you are at risk of failing, I will drop you from the course. I will provide 24 hours advance notice via Email.

MAKE-UPS: All submissions are due on the specified date and time (refer to the 'Lab Schedule') at the start of the lab, or as instructed by your TA. Assignments left at the Biology Office will not be accepted. Reports submitted at the end of the lab session will not be accepted either. Submissions after the due date will not be accepted unless otherwise stated by the instructor. Make-up opportunities for quizzes, lab sessions, or Experiment Progress Reports will not be provided unless pre-approved. If you know in advance that you'll miss lab due to a pre-scheduled activity, you must get permission from your TA at least one week prior to the class you'll miss.

CIVILITY STATEMENT: Please be respectful of all students' right to learn without disruptions. Please be kind and respectful to your classmates and most importantly use this lab to practice professional and ethical behavior.

CELL PHONES: All cell phones and tablets must be switched off or set to silent mode. Do not answer phone calls during class. Laptops are allowed for class-related purposes only; they must not be used for other activities unrelated to the class.

ACADEMIC DISHONESTY: Academic dishonesty in any form is strictly prohibited and will result in disciplinary action in accordance with University regulations. Refer to UTEP's Handbook of Operating Procedures, Chapter 1: Student Conduct and Discipline for more details.

PLAGIARISM: Plagiarism is a form of academic dishonesty that will not be tolerated. Visit UTEP's Library Plagiarism and Scholastic Integrity webpage for guidance.

ADA ACCESSIBILITY: "If you have or suspect a disability and need classroom accommodations, you should contact the Center for Accommodations and Support Services (CASS) at 747-5148." You can also e-mail the office at cass@utep.edu or go by their office in Union Building East (Room 106). For additional information, visit the CASS website at <http://sa.utep.edu/cass/>.

Students who have CASS accommodations need to meet and discuss with each course coordinator at the beginning of the semester and at least 1 week prior to using the accommodations.

MILITARY STATEMENT: Military students should promptly inform the instructor if they may be called to military service/training during the semester.

LAB SAFETY AND BIOSAFETY: All students must be certified and trained by Environmental Health and Safety in biosafety and lab safety procedures to participate in lab sessions. We will continually review safety procedures in the lab as well as has provided quizzes.

GUIDELINES AND STANDARD OPERATING PROCEDURES FOR WORKING IN THE LABORATORY

The laboratories planned for this semester are safe. However, we will be working with physical, biological, and chemical hazards that require safe handling conditions. **RAMP**, which stands for **Recognize, Assess, Minimize, and Prepare**, is a framework for laboratory safety that is widely promoted by various organizations and institutions involved in laboratory safety; thus it will be important to learn how to do the following when entering the lab:

- **Recognize** hazards
- **Assess** the risks of hazards
- **Minimize** the risks of hazards.
- **Prepare** for emergencies

Please take a few minutes to review these practices provided in the manual (LABORATORY 1: BIOSAFETY AND LABORATORY SAFETY TRAINING AT UTEP) prior to entering the lab. In the future, as you progress in your scientific training, you will encounter a variety of HAZARDOUS chemicals and biological substances. Therefore, it is essential that you develop good laboratory practices now. In general, if you are not sure of something, ask your instructor! It may prevent a problem. Carelessness and ignorance are the most common causes of personal injury in the laboratory. It is essential that a student follow the instructions given by your instructor.

GENERAL SAFETY EQUIPMENT AND MATERIALS

Personal protective clothing

- Gloves (provided by the laboratory)
- Gowns (Lab coat)
- Safety goggles

Decontamination

- Bleach for cultures
- 75% Ethanol for decontaminating surfaces

Containment

- Biosafety cabinets
- Culture materials
- Biosafety waste bags

GOOD LABORATORY PRACTICES RELATED TO YOUR PERSONAL SAFETY:

These guidelines outline essential practices to ensure your personal safety while working in the laboratory. By adhering to these guidelines, you actively contribute to a safer and more secure laboratory environment, safeguarding your well-being and that of your fellow participants.

1. **UNDERSTANDING HAZARDS:** Gain a comprehensive understanding of potential health and safety hazards associated with the equipment and chemicals you will handle. Consult Safety Data Sheets (SDS) provided by chemical manufacturers to assess potential risks and emergency response procedures. Review the SDS before entering the laboratory. You may find the university catalog of Safety Data Sheets here: [SDS Database link](#).

2. **SAFE HANDLING OF HAZARDOUS CHEMICALS:** Exercise caution when dealing with hazardous chemicals. Keep them covered and stationary, preferably towards the back of your lab bench, to minimize the risk of accidental spills. Unused chemicals must not be returned to their original containers. Avoid using unlabeled chemicals.
3. **AVOID INGESTION AND INHALATION:** Refrain from tasting or inhaling any materials within the lab setting. When applicable, conduct chemical work within the fume hood under your instructor's guidance.
4. **APPROPRIATE LAB ATTIRE:** Wear suitable clothing that adheres to safety standards. Closed-toed shoes are mandatory; open-toed footwear such as sandals or flip-flops is strictly prohibited. Personal Protective Equipment (PPE) is obligatory as directed by your instructor. Students must wear a lab coat (provided by the student), appropriate eye protection (provided by the student), and gloves (provided by the laboratory).
5. **SECURING LONG HAIR:** If you have long hair, secure it to prevent interference, contamination, or fire-related incidents.
6. **EMERGENCY PREPAREDNESS:** Familiarize yourself with the locations and proper usage of emergency equipment, including eyewash stations, fire extinguishers, exits, and other essential tools.
7. **PIPETTING CAUTION:** Never use your mouth for pipetting tasks. Utilize appropriate pipetting tools to handle liquids safely.
8. **NO FOOD OR DRINK:** Refrain from consuming food or beverages within the laboratory premises. This includes candy, gum and vapes.
9. **PROPER PPE REMOVAL:** Remove all PPE before exiting the lab.
10. **HAND HYGIENE:** Thoroughly wash your hands before leaving the lab. Even trace amounts of hazardous chemicals or biological materials on your hands can lead to unintended ingestion if not washed off before eating.
11. **MAINTAINING CLEAN WORK AREAS:** Keep your workspace orderly and sanitized. Dispose of chemicals only in designated, labeled containers meant for proper disposal. Avoid pouring any chemicals down sink drains.

EMERGENCY RESPONSE PROCEDURES

- If you are injured, notify the instructor IMMEDIATELY.
- If a chemical or biological spill occurs, notify the instructor IMMEDIATELY.
- If the fire alarm is activated, IMMEDIATELY leave the building with your instructor and await further instructions.
- Emergency contact numbers:

Contact	Phone #/ext	Address
University Police	ext. 5611	3118 Sun Bowl Drive
Main Office, Biological Sciences Department	ext. 5844	Bioscience Research Bldg., Rm. 2.120
Environmental Health and Safety	ext. 7124	EPNG Building, Room 10
Facilities Services Emergency	ext. 7187	3121 Sun Bowl Drive
Life Threatening Situations	911	

ASSIGNMENTS AND GRADING STRUCTURE

Some assignments for this course are assessed according to rubrics. You can find these rubrics by clicking on the appropriate assignment link in Blackboard and choosing to “View Rubric” from the button beneath the Points Possible for the assignment. Detailed rubrics for the research proposal, presentation and lab notebook are provided in the appendices of the lab manual.

GRADE DISTRIBUTION:

700-630	629-560	559-490	489-420	<419
A	B	C	D	F

Assignments	PTS
Research Proposal	200
Group Presentation	200
Quizzes	90
Homework/In-Lab Activities	110
Lab Notebooks	70
BIOSAFETY AND LAB SAFETY CERTIFICATIONS	30
Extra credit Surveys	10

SUMMARY OF GRADED ASSIGNMENTS

BIOSAFETY AND LAB SAFETY CERTIFICATIONS (INDIVIDUAL) 30PTS

Completion of online biosafety and lab safety training is mandatory for lab participation.

QUIZZES AND SURVEYS (INDIVIDUAL GRADE) 90 PTS

Every lab session concludes with an exit quiz, designed to assess your understanding of various lab aspects, including protocols, safety procedures, and the day's activities. These quizzes serve both as formative feedback for you and instructional feedback for the teaching team. Some quizzes may be online.

HOMEWORK ASSIGNMENTS (INDIVIDUAL GRADE) 110 PTS

There will be individual homework assignments due this semester

LABORATORY NOTEBOOK (INDIVIDUAL GRADE) 70 PTS

Each student is required to maintain a comprehensive lab notebook, accounting for 70 points of the laboratory grade. This notebook serves as an exhaustive record of protocols, discussions, experiments, and results across the semester. Emphasis should be placed on legibility, neatness, and meticulous organization. While handouts need not be replicated, they can be affixed to the appropriate sections. Collaborate with your lab partners while composing findings and other materials, but ensure that each exercise is documented in your own words. The act of plagiarism will not be tolerated. Random checks will be conducted on lab books.

RESEARCH PROPOSAL (TEAM GRADE) 200 PTS

Research proposals are essential components of the scientific process, outlining your plan to investigate a specific topic you have created based on your experiments, literature searches and what you have learned in your lecture.

FINAL POSTER PRESENTATION (TEAM GRADE) 200 PTS

The laboratory reports will be prepared in groups. It should contain title, objectives, methodology, results, discussion, and references.

CONSEQUENCES, MAKING MISTAKES VS FAILURE TO COMPLY WITH LAB RULES.

We generally try to avoid punitive measures to ensure compliance, however this doesn't mean that you won't be free of consequences. Making mistakes is essential to learning and our goal is to create a space with a certain latitude for messing-up. However, there are differences between mistakes and actions done out of complete negligence. That being

said, the following are non-negotiables and have immediate consequences resulting in grade reduction or reporting to the office of Academic integrity:

- Deliberate failure to comply with biosafety practices and procedures.
- Failure to come prepared with the appropriate PPE will also result in a grade reduction and loss of credit for the lab. No lab coat, no proper attire no entrance, no exceptions.
- Misconduct or horse-play in the lab.
- Likewise, disrespecting classmates or TA will involve the student's immediate removal from class. A grade of "0" will be given for any assignments missed.
- Missing labs without providing an excuse or communicating with the instructor of record and TA.
- Failure to turn in assignments.
- Ghosting- Repeated failure to show up and/or communicate with your teammates or instructor of record
- Cheating or plagiarizing on written assignments.

ACCOUNTABILITY AND LEARNING FROM MISTAKES

In this lab environment, your responsibility extends not only to adhering to biosafety protocols and the policies outlined in this syllabus, but also to your fellow team members. Our aim is to encourage you to exercise caution and diligence to prevent significant errors, while also recognizing the value of mistakes as learning opportunities. We encourage a balance that allows you to grow and improve without jeopardizing safety or progress. Both as a team and individually, errors are inevitable; however, taking certain steps can aid in recovery and advancement. This sentiment underscores our proactive approach to addressing errors, focusing not on avoiding responsibility, but on swiftly rectifying errors and improving how we work. The strategy is to stay ahead by acknowledging and resolving mistakes as promptly as possible. Keep this in mind as you practice proper lab safety and develop your communication skills with your lab mates about assignments.

Here's a suggested approach:

Admit and Take Responsibility: Be sincere but succinct, avoiding self-deprecation. For instance: "I apologize for the error I made in {explain}. I am currently working on rectifying it and will keep you updated on my progress." Your team will likely appreciate your proactive handling of the situation without an extended explanation.

IDENTIFY, INTERPRET, AND SAFEGUARD: This step should be done as a team, especially when there are important lessons to be learned. Pinpoint exactly where the mistake occurred. Probe into the root causes and develop measures to prevent its recurrence. By analyzing the error comprehensively, you can design policies and safeguards to ensure it doesn't happen again. Have some grace for yourself and with each other during this process.

INFORM, DESCRIBE, AND LEARN: Communicate with your team members about your corrective actions. Describe how you plan to address the issue and outline preventive measures to avoid its reoccurrence in the future. This practice fosters transparency, accountability, and collective learning.

Remember, our objective is not to eliminate mistakes entirely, but to promote a culture where errors are recognized, acknowledged, and used as catalysts for growth and improvement. Embracing a mindset of continuous learning ensures that you and your team progress effectively in your endeavors. It also helps to reduce the annoyances of shame or anxiety driven behavior.

TENTATIVE COURSE SCHEDULE

Week	Date	Activity	Reading & Preparation	Homework/Assignment	Quiz/Assessment	Due
1	27-28 Aug	ONLINE: Laboratory Safety/Biosafety Training/Introduction to Immunology Lab	Syllabus, Biosafety materials, Overview of lab	-	-	-
2	3-4 Sep	ONLINE: Lab Notebooks/The Scientific Method	Complete biosafety and lab safety training on Blackboard, The Scientific Method	Online certification quiz	Intake survey	Biosafety and Lab Safety Certificates and Intake Survey - Due 11:59p.m. Day before lab.
3	10-11 Sep	Elements of a Research Proposal/ Normal Peripheral Blood Smears	Elements of a Research Proposal & Examination of Peripheral Blood Smears	Lab coat, Safety glasses, Lab notebook	Quiz 1-Biosafety	-
4	17-18 Sep	Literature Review/ Diseased Peripheral Blood Smears	Review of research paper posted on Blackboard	Start online research with your team for Lab Report 1.	Quiz 2-Lab Notebooks	-
5	24-25 Sep	Sample Preparation, Culture, and Harvesting of Cells/ Culture of L929 and WEHI Cells	Cellular Techniques	-	Quiz 3-Scientific Literature	Research Proposal Lab Report 1 and Notebook due
6	1-2 Oct	ELISA – M-CSF and/or IL-3	Watch videos on ELISA	-	Quiz 4-Tissue Culture	-
7	8-9 Oct	Culture of 32Dcl3 Cells, Counting, and Addition of Cytokines	Watch videos on Cellular Techniques and Basic Math for the Laboratory	Online research with your team for Lab Report 2.	Quiz 5-ELISA	-
8	15-16 Oct	Harvest of 32Dcl3 Cells for Multiple Assays (e.g. RNA, cytopins for microscopy)	-	-	-	Research Proposal Lab Report 2 and Notebook due
9	22-23 Oct	Cellular Techniques II/ Wright-Geimsa Staining and Microscopy	Watch video on Wright-Giems staining	-	Quiz 6-Wright-Geimsa staining and microscopy	-
10	29-30 Oct	In lab proposal preparation/ Culture of 32Dcl3 Cells, Counting, and Addition of Cytokines	-	Online research with your team for Lab Report 2.	-	-

11	5-6 Nov	Flow Cytometry I / CD11b Staining of 32Dcl3 Cells Undergoing Myeloid Differentiation	Watch Videos on flow cytometry	-	Quiz 7 -Flow Cytometry	Research Proposal Lab Report 3 and Notebook due
12	12-13 Nov	Flow Cytometry II / Annexin V Staining of 32Dcl3 Cells Upon IL-3 Withdrawal	Watch videos on Apoptosis	Online research with your team for Lab Report 3.	Quiz 8 -Apoptosis	
13	19-20 Nov	Molecular Biology Techniques/ RNA Extraction for RT-qPCR	Watch Videos on RT-qPCR	-	Quiz 9 -qRT-PCR/PCR	-
14	26-27 Nov	THANKSGIVING – NO CLASS	-	-	-	-
15	3-4 Dec	FINAL PRESENTATIONS	-	Final Presentations	-	Research Proposal Lab Report 4 and Notebook due
16	10-11 Dec	NO CLASS!!!!	-			

LABORATORY 1 (VIRTUAL): BIOSAFETY AND LABORATORY SAFETY TRAINING AT UTEP

Environmental Health and Safety (EHS) programs are vital for ensuring the health and safety of employees, the public, and the environment in the workplace. The main objective is to prevent injuries, illnesses, and environmental harm. It is the responsibility of everyone to adhere to the rules and regulations set by the department and the University to ensure community safety. To participate in this lab throughout the semester, you need to complete online training and obtain certification from EH&S.

Biosafety training is an ongoing process that involves constant assessment and certification. For each new experiment, project, protocol, or procedure, researchers must assess safety and plan accordingly. RAMP, which stands for Recognize, Assess, Minimize, and Prepare. It is a framework for laboratory safety that is widely promoted by various organizations and institutions involved in laboratory. It represents a set of best practices and principles that have been endorsed and adopted by the scientific and laboratory safety community. While the labs planned for this semester are safe, they involve physical, biological, and chemical hazards that require proper handling. Therefore, it's crucial to learn the following when entering the lab:

1. **Recognize:** This step involves identifying and recognizing potential hazards in the laboratory. It's about being aware of the risks associated with various experiments, chemicals, equipment, and procedures. It includes hazard identification and risk assessment.
2. **Assess:** Once hazards are recognized, the next step is to assess the risks associated with those hazards. This involves evaluating the severity of potential harm and the likelihood of accidents or incidents occurring. Risk assessment helps prioritize safety measures.
3. **Minimize:** After identifying and assessing risks, the goal is to minimize or control those risks. This step involves implementing safety measures and practices to reduce the likelihood of accidents. This can include using personal protective equipment (PPE), following established protocols, and engineering controls.
4. **Prepare:** Preparation is essential for responding to emergencies. This step involves having a plan in place for dealing with accidents, spills, fires, or other unexpected events. It includes knowing the location of safety equipment, having emergency contacts, and knowing the proper procedures for handling emergencies.

Safety is an integral part of laboratory work, and every researcher must have proactive mindset in identifying and mitigating potential hazards. Please take a few minutes to review these practices in the manual before entering the lab. As you progress in your scientific training, you'll encounter hazardous chemicals and biological substances. Developing good laboratory practices now is essential. If unsure, always ask your instructor; it could prevent potential issues. Carelessness and lack of knowledge are common causes of injuries in the lab. It's vital to follow the guidance from your instructor.

GENERAL SAFETY EQUIPMENT AND MATERIALS USED IN THE IMMUNOLOGY TEACHING LAB

Personal protective clothing

- Gloves (provided by the laboratory)
- Gowns (Lab coat)
- Safety goggles

Containment

- Biosafety cabinets.
- Culture materials
- Biosafety waste bags

Decontamination

- Bleach for cultures
- 75% Ethanol for surface decontamination

Please be mindful of the fact that PPE, Containment and Decontamination Policies will vary depending the Biosafety level of the lab and nature of research, it is your responsibility and that of the Principle Investigator/Lab Manager to either learn and/or share that information with lab members. Don't assume, make sure you ask and learn proper procedures for each lab.

GOOD LABORATORY PRACTICES RELATED TO YOUR PERSONAL SAFETY:

These guidelines outline essential practices to ensure your personal safety while working in the laboratory. By adhering to these guidelines, you actively contribute to a safer and more secure laboratory environment, safeguarding your well-being and that of your fellow participants.

- 1. UNDERSTANDING HAZARDS:** Gain a comprehensive understanding of potential health and safety hazards associated with the equipment and chemicals you will handle. Consult Safety Data Sheets (SDS) provided by chemical manufacturers to assess potential risks and emergency response procedures. Review the SDS before entering the laboratory.
- 2. SAFE HANDLING OF HAZARDOUS CHEMICALS:** Exercise caution when dealing with hazardous chemicals. Keep them covered and stationary, preferably towards the back of your lab bench, to minimize the risk of accidental spills. Unused chemicals must not be returned to their original containers. Avoid using unlabeled chemicals.
- 3. AVOID INGESTION AND INHALATION:** Refrain from tasting or inhaling any materials within the lab setting. When applicable, conduct chemical work within the fume hood under your instructor's guidance.
- 4. APPROPRIATE LAB ATTIRE:** Wear suitable clothing that adheres to safety standards. Closed-toed shoes are mandatory; open-toed footwear such as sandals or flip-flops is strictly prohibited. Personal Protective Equipment (PPE) is obligatory as directed by your instructor. Students must wear a lab coat (provided by the student), appropriate eye protection (provided by the student), and gloves (provided by the laboratory).
- 5. SECURING LONG HAIR:** If you have long hair, secure it to prevent interference, contamination, or fire-related incidents.
- 6. EMERGENCY PREPAREDNESS:** Familiarize yourself with the locations and proper usage of emergency equipment, including eyewash stations, fire extinguishers, exits, and other essential tools.
- 7. PIPETTING CAUTION:** Never use your mouth for pipetting tasks. Utilize appropriate pipetting tools to handle liquids safely.
- 8. NO FOOD OR DRINK:** Refrain from consuming food or beverages within the laboratory premises. This includes candy, gum and vapes.
- 9. PROPER PPE REMOVAL:** Remove all PPE before exiting the lab.
- 10. HAND HYGIENE:** Thoroughly wash your hands before leaving the lab. Even trace amounts of hazardous chemicals or biological materials on your hands can lead to unintended ingestion if not washed off before eating.
- 11. MAINTAINING CLEAN WORK AREAS:** Keep your workspace orderly and sanitized. Dispose of chemicals only in designated, labeled containers meant for proper disposal. Avoid pouring any chemicals down sink drains.

EMERGENCY RESPONSE PROCEDURES

- If you are injured, notify the instructor IMMEDIATELY.
- If a chemical or biological spill occurs, notify the instructor IMMEDIATELY.
- If the fire alarm is activated, IMMEDIATELY leave the building with your instructor and await further instructions.
- Emergency contact numbers:

Contact	Phone #/ext	Address
University Police	ext. 5611	3118 Sun Bowl Drive
Main Office, Biological Sciences Department	ext. 5844	Bioscience Research Bldg., Rm. 2.120
Environmental Health and Safety	ext. 7124	EPNG Building, Room 10
Facilities Services Emergency	ext. 7187	3121 Sun Bowl Drive
Life Threatening Situations	911	

INTRO TO BIOSAFETY

Biosafety aims to prevent significant biological disruptions that affect ecology and human health. It covers ecology, agriculture, medicine, chemistry, exobiology, and addresses future biosafety challenges. For high-risk threats, the emerging field of biosecurity is crucial.

LABORATORY BIOSAFETY LEVELS

The CDC and NIH outline four biosafety levels (BSL) that correspond to activities involving infectious agents. These levels are categorized from least to most protective measures provided to laboratory personnel, the environment, and the community. BSL1 pertains to infectious agents with minimal or no hazards, while BSL4 pertains to agents posing the highest risk. Each level involves specific facility design, laboratory practices, and safety equipment tailored to the infectious agent being handled. Below, we briefly discuss BSL1 through BSL4. For an in-depth examination of biosafety level criteria, refer to CDC/NIH's publication "Biosafety in Microbiological and Biomedical Laboratories." The pathogens studied in the Immunology Laboratory (MICR 4354) wet labs fall under Biosafety Level 1 and Biosafety Level 2 organisms. However, BSL-2 practices and procedures will be employed.

BIOSAFETY LEVEL 1 (BSL-1)

Biosafety Level 1 (BSL-1) standard practices, safety equipment, and facility specifications are generally appropriate for undergraduate and secondary educational training and teaching laboratories and for other laboratories that work with defined and characterized strains of viable biological agents not known to consistently cause disease in healthy adult humans.² BSL-1 offers basic protection and is suitable for work involving characterized strains of viable biological agents that do not typically cause disease in healthy adults. BSL-1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended. BSL-1 represents a basic level of containment that relies on standard, microbiological best practices and procedures with no special primary or secondary barriers, other than a door, a sink for handwashing, and non-porous work surfaces that are cleanable and easy to decontaminate.¹

BIOSAFETY LEVEL 2 (BSL-2)

Biosafety Level 2 (BSL-2) standard practices, safety equipment, and facility specifications are applicable to laboratories in which work is performed using a broad-spectrum of biological agents and toxins that are associated with causing disease in humans of varying severity. With good practices and procedures, these agents and toxins can generally be handled safely on an open bench, provided the potential for producing splashes and aerosols is low. The primary hazards to lab personnel working with these agents include accidental skin or mucous membrane exposures, or ingestion of infectious materials. BSL-2 is appropriate when work is done with any human-derived blood, body fluids, or tissues where the presence of an infectious agent may be unknown. **Primary barriers** recommended include **biological safety cabinets (BSC)** and **personal protective equipment (PPE)**. **Secondary barriers** recommended include **waste decontamination facilities**. Extreme caution should be taken with contaminated needles and other sharp materials. Waste decontamination capabilities to reduce the potential of environmental contamination and the separation of laboratory spaces from office and public spaces to reduce the risk of exposure to other personnel should be considered. BSL-2 is designed for handling moderate-risk agents causing human diseases of varying severity through ingestion, skin exposure, or mucous membrane contact. Our focus will primarily be on BSL-1 agents with some BSL-2. All lab biosafety practices will follow BSL-2 standards.

BIOSAFETY LEVEL 3 (BSL-3)

BSL-3 practices, safety equipment, and facilities are recommended for clinical, diagnostic, research, or teaching laboratories involving indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. Primary hazards to lab personnel working with these agents include autoinoculation, ingestion, and exposure to infectious aerosols. Primary barriers that must be used include BSCs or other

enclosed equipment. Secondary barriers for this level include controlled access to the laboratory, a specialized ventilation system, and waste decontamination facilities.

BIOSAFETY LEVEL 4 (BSL-4)

BSL-4 represents maximum containment and is required for dangerous and exotic agents that pose a high risk of life-threatening disease. These are agents that may be transmitted via the aerosol route and for which there is no available vaccine or therapy.

BIOLOGICAL SAFETY CABINETS (BSC'S)

Biological safety cabinets (BSC's) are among the most effective, as well as the most commonly used, primary containment devices in laboratories working with infectious agents. The BSC is designed to capture and contain any infectious particulates or aerosols generated within the BSC's interior and exhaust them through a high-efficiency particulate air (HEPA) filter either into the laboratory, or to the outside.

The three general types of BSC's are available (Class I, II, and III). More detailed information on BSCs can be found in CDC/NIHs "Biosafety in Microbiological and Biomedical Laboratories." The BSC's available to us in Room B412 of the Biology Building are Class II, Type A2. The Class II BSC has an increased face velocity relative to the Class I BSC and the additional advantage of providing protection to the research material by HEPA filtration of the airflow into the cabinet across the work surface. This type of cabinet will protect the user, environment, and the research material and is suitable for work with moderate- to high-risk agents (in the case of our laboratory, Biosafety Level 2). Class II BSCs are classified into two types: A & B. Type A cabinets like ours exhaust the cabinet air into the laboratory. Since the air is re-circulated within the laboratory, volatile or toxic chemicals and radionuclides should not be used inside this type of cabinet. A diagram of airflow circulating in a Class II, Type A BSC is pictured in **Figure 1**.

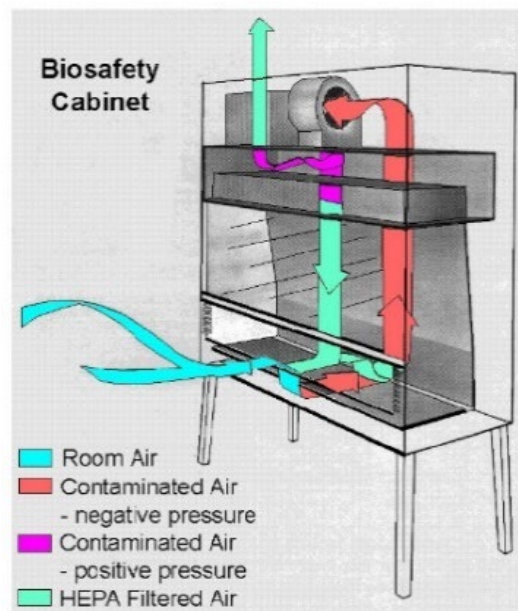


FIGURE 1. SCHEMATIC OF AIRFLOW CIRCULATION IN A CLASS II, TYPE A BIOSAFETY CABINET.

STANDARD OPERATING PROCEDURES FOR THE BIOSAFETY CABINETS: PROPER USE OF BIOLOGICAL SAFETY CABINETS

As with any other piece of laboratory equipment, personnel must be trained in the proper use of the BSC. Strict adherence to recommended practices for the use of the BSC is important in attaining the maximum containment capability of the equipment and maintaining mechanical performance of the equipment itself.

PROPER USAGE OF PATHOGENIC ORGANISMS AND INFECTIOUS MATERIALS IN THE BSC:

To ensure safe handling of pathogenic organisms and potentially infectious materials within the Biological Safety Cabinet (BSC), follow these crucial steps:

1. Preparation and Initiation:

- Run the BSC blower for 15 minutes before starting work to clear airborne contaminants.
- Disinfect the BSC work surface with either 70% ethanol or a freshly prepared 10% chlorine bleach solution. Note that bleach can damage stainless steel and requires thorough water washing to prevent corrosion.
- Arrange your work area, including a biohazard bag for solid waste and a container with disinfectant for liquid waste. Keep a disinfectant squirt bottle accessible for potential spills. Do not use open flames; a ceramic hot plate is provided for drying samples on slides before Gram staining. Set up any necessary slides, tubes, or plates before starting work.

2. Proper Setup:

- Avoid placing objects on air intake or exhaust grills to maintain airflow.
- Conduct all work at least four inches from the BSC opening.
- Wear necessary personal protective equipment (PPE), such as lab coat, eye protection, gloves, etc., as instructed by your TA.

3. Working in the BSC:

- Perform tasks within the BSC with deliberate, careful movements to minimize disruption of airflow.
- Report any spills immediately, inside or outside the BSC, to your instructor. Follow instructor guidance for proper cleanup.

4. Disposal and Completion:

- Dispose of used materials, especially those in contact with biological agents, properly in designated containers.
- Run the BSC blower for five minutes after completing work to clear airborne contaminants.
- Do not open plates or tubes containing pathogenic organisms outside the BSC.
- Remove PPE, thoroughly wash hands and arms before leaving the lab.

5. Additional Containment Considerations:

- Refrain from activities that may disrupt the inward directional airflow through the work opening of the BSC. The following may result in escape of aerosolized particles from within the containment unit:
 1. Repeated insertion and withdrawal of the workers' arms into and out of the work chamber,
 2. opening and closing doors to the laboratory,
 3. improper placement or operation of materials or equipment within the work chamber, or
 4. Brisk walking past the BSC while it is in use are demonstrated causes of the escape of aerosolized particles from within the cabinet.

STANDARD OPERATING PROCEDURES FOR THE ENTIRE LAB:

These procedures will be updated as we begin to work with microorganisms and cell cultures in the lab. Part of your participation grade will be based on your ability to follow these procedures. Beginning the week of September 18th, you will be asked to leave all PPE in the lab to minimize outside exposure of biohazardous materials. We will provide services to clean lab coats prior to the last week of classes

- Spray and wipe down the benchtop area with 70% ethanol before and after work.
- Dispose of materials correctly: used tips, micro-centrifuge tubes, and disposable loops in designated boxes or bags; serological pipettes in the long cardboard box; used plates, conical tubes, gloves, and petri dishes in the red biohazard bin.
- Place clean broken glass in the labeled cardboard box; used glass microscope slides in the provided container.
- Discard used tips in the designated biohazard waste box or small bag when working on the benchtop. Avoid disposing of gloves, glass, or paper towels there.
- Empty biohazard bins within the BSC when they are three-quarters full, and place bags in the larger biosafety waste container.
- Wash hands after removing gloves and before leaving the lab.
- Inform your TA of any spills in the hood or lab.

INDIVIDUAL HOMEWORK: ONLINE BIOSAFETY AND LAB SAFETY TRAINING.

1. Review the University's [Environmental Health and Safety page](#).
2. The following pages are available for your review and are valuable references for conduct in the lab:
 - a. [Laboratory Safety](#)
 - b. [SDS Database](#)
3. Receive and certification for the following (note-you will need to log in with your UTEP NetId and Password):
 - a. The Main portal link is [here](#). We ask that you take the online course from EHS [Biosafety and Bloodborne Pathogens \(online\)](#)
 - b. [Laboratory Safety for BIO/COHS/PSYC/PHARM \(online\) class](#)
4. Once you've completed your training and received your certification upload it to the **link provided on Blackboard**. Print your certificates and put it into your lab notebook!

LABORATORY 2 (VIRTUAL): LAB NOTEBOOKS & THE SCIENTIFIC METHOD

LAB NOTEBOOKS

The laboratory notebook or research notebook is one of the most important tools for documenting and communicating scientific research, discovery, and experimentation. More importantly, the laboratory notebook is NOT a work of fiction, it is used not only to document, record, and organize all aspects of laboratory work and research, but is essential for maintaining scientific integrity and ethics. They should document the complete and accurate history of research, helping to prevent data manipulation or fraud.

Data Record Keeping: The central purpose of a lab notebook is to record experimental data, observations, procedures, and outcomes. It provides a systematic and organized way to document every detail of an experiment, from the initial setup to the final results.

Legal and Intellectual Property Protection: Lab notebooks are considered legal documents and can serve as evidence in patent applications, disputes, or legal matters related to research findings. They help establish ownership of intellectual property and innovations.

Replicability and Verification: Detailed and well-maintained lab notebooks enable other researchers (or even your future self) to replicate experiments and verify results. This is fundamental to the scientific method and ensures the reliability of research.

Record of Failures and Successes: A lab notebook doesn't just document successful experiments; it also records failed attempts, unexpected outcomes, and mistakes. This information can be invaluable in troubleshooting and learning from past experiences.

Research Progress Tracking: Researchers use lab notebooks to track the progress of their work. It helps in setting goals, planning experiments, and monitoring the advancement of research projects over time.

Communication Tool: Lab notebooks can be shared among team members and collaborators. They facilitate communication by providing a comprehensive account of ongoing experiments, allowing others to understand and contribute to the research.

Quality Control: In regulated industries and research settings, lab notebooks play a critical role in quality control and compliance. They help ensure that research is conducted according to established protocols and standards.

Ethical Standards: Lab notebooks are essential for maintaining scientific integrity and ethics. They document the complete and accurate history of research, helping to prevent data manipulation or fraud.

Documentation of Methods: Detailed descriptions of experimental methods and procedures are crucial for replicating research. This information allows others to build on your work and contributes to the cumulative knowledge of the scientific community.

Innovation and Creativity: Lab notebooks are not limited to data and procedures. They also serve as a platform for brainstorming ideas, sketching diagrams, and jotting down insights, fostering creativity and innovation.

Long-Term Reference: Lab notebooks are often retained for many years, serving as a long-term reference for future research, publications, or patent applications. They provide a historical record of scientific work.

Personal Learning Tool: Keeping a lab notebook encourages researchers to reflect on their work, learn from their mistakes, and refine their experimental techniques. It aids in the continuous improvement of research skills.

In summary, a laboratory notebook is a fundamental and multifunctional tool in scientific research. It ensures the transparency, reproducibility, and reliability of experiments, while also serving as a legal document, a communication medium, and a repository of scientific knowledge. Properly maintained lab notebooks contribute to the rigor and credibility of scientific research.

Each student is expected to maintain a detailed lab notebook, contributing 20 points to the laboratory grade. This lab book serves as an elaborate record of protocols, discussions, experiments, and results relevant to each lab exercise undertaken throughout the semester. Legibility, neatness, and meticulous organization are important for both long-term referencing and communicating to others your work. While it is unnecessary to transcribe handouts or protocols from your lab manual, they can be integrated through cutting, pasting, or taping into the designated sections. However, it is important to keep notes of modifications performed on any pasted material. Collaborate with your lab partners to document and summarize findings and materials, ensuring that the writing of each exercise remains in your distinct voice. Plagiarism is sternly prohibited. Lab books will be subject to random review. Submission of completed lab books is anticipated in-class on the final day. A comprehensive Rubric detailing the assessment of your laboratory notebook is provided below. It is crucial to peruse this section attentively. Your notebook for each lab exercise should encapsulate the subsequent components:

I. Title:

Succinct and descriptive, for example "Handling of Animals, Immunizations."

II. Introduction:

Elucidating the purpose of the lab exercise or experiment, encompassing background information presented by your instructor or included in handouts. A minimum of two paragraphs is expected.

III. Materials and Methods:

Detailing the supplies, reagents, equipment employed, and the procedure executed for the experiment. This section should be comprehensive enough to facilitate the replication of your work by others.

IV. Results:

Offering a documented account of observations, findings, and raw data. This segment should also incorporate data in the form of drawings, graphs, or tables, where pertinent. Ensure that these visual aids are numbered and equipped with concise titles.

V. Discussion:

Summarizing insights gleaned from both methodological and principal perspectives. Additionally, address encountered challenges and propose potential solutions.

LABORATORY NOTEBOOK RUBRIC

Criteria	Excellent (4)	Good (3)	Satisfactory (2)	Needs Improvement (1)	Unsatisfactory (0)
Title	Titles for each lab exercise are clear, concise, and accurately reflect the content of the exercise.	Most titles are relevant to the content, but a few may lack clarity or conciseness.	Titles are present, but some are unclear or do not accurately represent the exercise.	Titles are often missing or lack clarity, making it difficult to identify the lab exercise.	Titles are consistently missing for multiple lab exercises.
Introduction	Introduction is well-written, provides a clear purpose for the lab exercise, and includes comprehensive background information. Minimum of 2 paragraphs is met with insightful content.	Introduction provides a purpose for the lab exercise and includes some background information. May lack depth or clarity in a few places. Minimum of 2 paragraphs is met.	Introduction briefly states the purpose of the lab exercise but lacks sufficient background information. Minimum of 2 paragraphs may not be met.	Introduction lacks clarity or depth, making it challenging to understand the purpose of the lab exercise. Falls short of the minimum of 2 paragraphs.	Introduction is absent or severely lacking, making it difficult to understand the purpose of the lab exercise.
Materials and Methods	Detailed description of supplies, reagents, equipment, and procedure is provided. It's clear, well-organized, and allows easy reproducibility.	Description of supplies, reagents, equipment, and procedure is mostly detailed and organized. Some areas may lack clarity or conciseness.	Description of supplies, reagents, equipment, and procedure is present but lacks sufficient detail or clarity in parts.	Description of supplies, reagents, equipment, and procedure is rudimentary and lacks clarity, making it difficult to reproduce the work.	Materials and methods are missing or severely insufficient.
Results	Observations, findings, and raw data are presented clearly and comprehensively. Data is well-organized and includes appropriate drawings, graphs, or tables when relevant.	Observations, findings, and raw data are clear, but some aspects may be lacking in detail or clarity. Data presentation is generally organized.	Observations, findings, and raw data are presented, but significant detail is missing or unclear. Data presentation lacks organization or completeness.	Observations, findings, and raw data are rudimentary and lack clarity. Data presentation is disorganized or inadequate.	Results section is incomplete, missing key data or explanations, making it challenging to understand the findings.
Discussion	Summary of what was learned from both methodological and principle standpoints is insightful and thorough. Problems encountered and possible solutions are discussed thoughtfully.	Summary of what was learned from methodological and principle standpoints is adequate, but may lack depth in places. Discussion of problems and solutions is present.	Summary of what was learned is brief, lacking depth or insight. Discussion of problems and solutions may be superficial.	Summary of what was learned is minimal and lacks detail. Discussion of problems and solutions is insufficient.	Discussion is absent or severely lacking, failing to address what was learned and problems encountered.

Criteria	Excellent (4)	Good (3)	Satisfactory (2)	Needs Improvement (1)	Unsatisfactory (0)
Legibility, Neatness, and Organization	Notebook is extremely neat, legible, and well-organized, enhancing its usability.	Notebook is generally neat and legible, with a few minor organizational issues.	Notebook is mostly neat and legible, but may contain some disorganized sections.	Notebook's legibility and neatness are occasionally compromised, affecting overall presentation.	Notebook is consistently messy, illegible, or disorganized, making it challenging to navigate and assess.
Collaboration and Plagiarism	All work is completed in your own words. Collaboration with lab partners is evident, and proper attribution is given for shared work. Plagiarism is not present.	Most work is in your own words, but there are a few instances of unclear attribution or possible collaboration without proper documentation. Plagiarism is minor or absent.	Some work appears to be copied directly from other sources without proper attribution or paraphrasing. Plagiarism is noticeable in parts.	Significant portions of work are copied from other sources without proper attribution or paraphrasing. Plagiarism is evident and substantial.	Widespread and blatant plagiarism is present throughout the notebook.

THE SCIENTIFIC METHOD

The **scientific method** is used to solve problems and explain phenomena. The development of the scientific method coincided with changes in philosophy underpinning scientific discovery, radically transforming the views of society about nature. During the European Renaissance, individuals such as Francis Bacon, Galileo, and Isaac Newton formalized the concept of the scientific method and put it into practice. Although the scientific method has been revised since its early conceptions, much of the framework and philosophy remains in practice today.

Step 1: The Observation and Question

Prior to investigation, a scientist must define the question to be addressed. This crucial first step in the scientific process involves observing some natural phenomena of interest. This observation should then lead to a number of questions about the phenomena. This stage frequently requires background research necessary to understand the subject matter and past work on similar ideas. Reviewing and evaluating previous research allows scientists to refine their questions to more accurately address gaps in scientific knowledge. Defining a research question and understanding relevant prior research will influence how the scientific method is applied, making it an important first step in the research process.

An everyday example: You are trying to get to school or work and your car won't start. The thought process that most people go through in that situation clearly mirrors the official scientific method (after you are finished getting upset). First, you make an observation: my car won't start! The question that follows: why isn't it working?

Step 2: The Hypothesis

The next step is making a hypothesis, based on prior knowledge. A hypothesis is an "uncertain explanation" or an unproven conjecture that seeks to explain some phenomenon based on knowledge obtained while executing subsequent experiments or observations. Generally, scientists develop multiple hypotheses to address their questions and test them systematically.

All hypotheses must meet certain criteria for the scientific process to work. First, a hypothesis must be testable and falsifiable. This aspect of the hypothesis is critical and of much greater importance than the hypothesis being correct. A testable hypothesis is one that generates testable predictions, addressed through observations or experiments. A falsifiable hypothesis is one that, through observation of conflicting outcomes, can be proven wrong. This allows investigators to gain more confidence over time, not by accumulating evidence showing that a hypothesis is correct, but rather by showing that situations that could establish its falsity do not occur.

Hypotheses come in two forms: null hypotheses and alternative hypotheses. The null hypothesis is tested against the alternative hypothesis and reflects that there will be no observed change in the experiment. The alternative hypothesis is generally the one described in the previous two paragraphs, also referred to as the experimental hypothesis. The alternative hypothesis is the predicted outcome of the experiment. If the null hypothesis is rejected, then this builds evidence for the alternative hypothesis.

An everyday example: Maybe it is freezing outside and therefore it is fairly likely that your car battery is dead. Maybe you know you were low on gas the night before and therefore it is likely that the tank is empty.

Step 3: Experimentation and Data Collection

Either way, the next step is to make more observations or to conduct experiments leading to conclusions. Following the formulation of hypotheses, scientists plan and conduct experiments to test their hypotheses. These experiments provide data that will either support or falsify the hypothesis. Data can be collected from quantitative or qualitative observations. Qualitative information refers to observations that can be made simply using one's senses, be that through sight, sound, taste, smell, or touch. In contrast, quantitative observations are ones in which precise measurements of some type are used to investigate one's hypothesis.

An experiment is a procedure designed to determine whether observations of the real world agree with or refute the derived predictions in the hypothesis. If evidence from an experiment supports a hypothesis, that gives the hypothesis more credibility. This does not indicate that the hypothesis is true, as future experiments may reveal new information about the original hypothesis. Experimental design is another critical step in the scientific method and can have a great effect on the results and conclusions one draws from an experiment. Careful thought and time should be devoted to experimental design and minimizing possible errors. The experiment should be designed so that every variable or factor that could influence the outcome of the experiment be under control of the researcher. Two types of variables are used to describe the conditions in an experiment: the independent and the dependent, or response, variable. The independent variable is directly manipulated or controlled by the scientist and is generally what one predicts will affect the dependent variable. The dependent, or response, variable thus depends on the value of the independent variable. Experiments are generally designed so that one specific factor is manipulated in the experiment in order to illuminate cause and effect relationships.

An everyday example: Does the car still have all of its parts? Is this the right key? What does the gas gauge say? Does a jump start help?

Another important aspect in experimental design is the role of the control treatment, which represents a non-manipulated treatment condition. The control treatment is kept in the same conditions as the experimental treatment, but the experimental manipulation is not applied to the control. For example, if a researcher were testing the effects of soil salinity on plant growth, the soil in the control treatment would have no added salt. The control provides a baseline of "normal" conditions with which to compare the experimental treatments.

Experimental design should also incorporate replicates of each treatment. Repeatability of experimental results is an important part of the scientific method that ensures the validity and accuracy of data. It is quite difficult to control all aspects of an experiment so there is inherent variation in results that cannot be controlled for even under the most carefully designed and controlled experiments. Having replicates enables an investigator to estimate this inherent variation in results. Precise recording and measurement of data is also of great importance for ensuring the accuracy of results and the conclusions one draws from the results.

Step 4: Results and Data Analysis

The next step in the scientific method involves determining what the results from the experiment mean. Scientists compare the predictions of their null hypothesis to that of their alternative hypothesis to determine if they are able to reject the null hypothesis. Rejecting the null hypothesis means that there is a significant probability that values of the dependent variable in the control versus experimental treatments are not equal to each other. If significant differences exist, then one can reject the null hypothesis and accept the alternative hypothesis. Conversely, the investigator may fail to reject the null hypothesis, meaning the treatment has no effect on the results. Before scientists can make any claims about their null hypothesis from their experimental data or observations, statistical tests are required to ensure the validity of the data and further interpretation of the data. Statistical tests allow researchers to determine if there are

genuine differences between the control and experimental treatments. From there, they can create figures and tables to illustrate their findings.

Step 5: Conclusions

The last portion of the scientific method involves providing explanations of the results and the conclusions that can be logically drawn from the results. Generally, this step of the scientific process also requires one to revisit scientific literature and compare their results with other experiments or observations on related topics. This allows researchers to put their experiment in a more general context and elaborate on the significance of particular results. Additionally, it allows them to explain how their work fits into a larger context in their discipline.

The scientific process does not stop here! The scientific process works through time as knowledge on topics in science accumulate and drive our understanding of particular mechanisms or processes explaining natural phenomena. If we fail to reject our null hypothesis, then it becomes necessary to revisit the initial stages of the scientific method and try to reformulate our questions and understand why an anticipated result was not attained.

Application of the Scientific Method

The only difference between the use of this method in every-day life and in the laboratory is that scientists carefully document their work, from observation to hypothesis to experiment, and finally conclusions and peer review. In addition, unlike problem solving outside the lab, the scientific method in the lab includes controlled conditions and variables.

LABORATORY 3 (IN PERSON): ELEMENTS OF A RESEARCH PROPOSAL & EXAMINATION OF NORMAL PERIPHERAL BLOOD SMEARS

ELEMENTS OF A RESEARCH PROPOSAL

A research proposal typically includes a title, abstract, introduction, literature review, methodology, and conclusion, along with a budget and timeline. It should also clearly define the research question, objectives, and any hypotheses. A successful proposal effectively communicates the significance of the study and the proposed approach to conducting it.

Here's a more detailed breakdown of the key elements:

Title and Abstract: Your proposal should start with a clear and concise title that accurately reflects the focus of your research. Your abstract should summarize the proposal, including the research question, methodology, and expected outcomes. This section should be written in a way that captures the reader's attention and encourages them to read on.

Introduction: Your introduction should provide a brief overview of the research topic, highlight the research gap or problem you intend to address, and explain why it is important to conduct this research. It should also provide a brief literature review that supports your research question and hypothesis.

Research Question and Hypothesis: Your research question should be specific, clear, and focused. It should guide the research process and should be framed in a way that suggests the potential for new insights or understanding. Your hypothesis should be testable and supported by your literature review.

Methodology: Your methodology should describe the research design, data collection, and data analysis methods you plan to use. It should explain why these methods are appropriate for your research question and hypothesis and should demonstrate your understanding of research ethics and best practices.

Significance and Expected Outcomes: Your proposal should describe the expected outcomes of your research, including the potential impact on the field and the potential contributions to theory, practice, and policy. You should also explain how your research will fill the research gap or problem you identified in your introduction.

Timeline and Budget: Your proposal should include a timeline that outlines the various stages of your research project and a budget that outlines the costs associated with each stage. Your budget should be realistic, and you should be prepared to justify each expense.

Conclusion: Your conclusion should summarize the key points of your proposal and reiterate the importance of your research. You should also explain why you are the best candidate to conduct this research, including your qualifications, experience, and skills.

RESEARCH PROPOSAL GUIDELINES

Whether you are new to writing research proposals or have some experience, here is a comprehensive guide to help you draft an effective proposal for an Immunology lab.

1. Introduction

A research proposal is a roadmap for your investigation. Begin with a concise but engaging introduction that highlights the importance of your chosen topic in the field of Immunology. Clearly state your research question or hypothesis and explain why it's worth exploring.

2. Literature Review (Background)

This section demonstrates your understanding of the existing research in Immunology. Summarize relevant studies, theories, and findings related to your topic. Identify gaps or unanswered questions that your research aims to address. Use reference management tools like Zotero or Mendeley to organize your sources and citations.

3. Hypothesis

Present your hypothesis, which is an educated guess about the outcome of your research. State how you expect your experiments to turn out based on your understanding of Immunology.

4. Aims and Objectives

Specify the goals of your research. Clearly define your primary and secondary objectives. These should be specific, measurable, achievable, relevant, and time-bound (SMART). Explain how achieving these objectives will contribute to the broader knowledge of Immunology.

5. Research Design and Methods

Detail the methodology you will use to conduct your research. Explain the experimental design, sample size, data collection methods, and data analysis techniques. If applicable, mention any ethical considerations and the steps you will take to ensure participant rights and confidentiality.

6. Expected Results

Outline the potential outcomes of your research. This could include possible findings that support or contradict your hypothesis. Discuss the significance of these outcomes and how they contribute to the field.

7. Conclusion

Summarize the main points of your proposal and reiterate its significance in advancing Immunology research. Conclude with a statement about your commitment to conducting the proposed study and contributing to the field.

8. Timeline

Create a timeline that outlines the key stages of your research, from literature review to data analysis and report writing. This helps you manage your project efficiently and ensures you allocate enough time to each step.

9. Budget

If applicable, outline the resources you will need for your research, including materials, equipment, and any funding required. Justify each expense and ensure your budget is realistic and accurate.

10. References

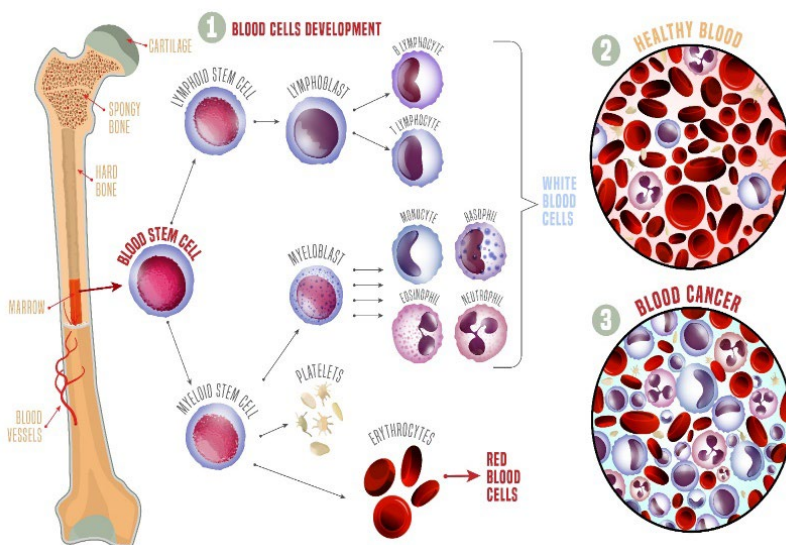
List all the references you cited in your proposal, following a consistent citation style (e.g., APA). Ensure that each reference is accurate and complete.

11. Proofreading and Revision

After drafting your proposal, proofread it carefully for grammar, spelling, and clarity. Seek feedback from your instructor of record, your teaching assistant, or peers to ensure your proposal is well-structured and effectively conveys your ideas.

Remember, writing a research proposal is a dynamic process that requires careful planning and thoughtful execution. By following this guide, you'll be well-prepared to craft a compelling research proposal for your Immunology lab.

EXAMINATION OF PERIPHERAL BLOOD SMEARS



INTRODUCTION

Immunology focuses on the immune system and its response to infections and diseases, whereas **hematology** is concerned with the study of blood and blood-related disorders. While there is some overlap, these fields have distinct areas of expertise and applications in healthcare and research. Blood tests, microscopy, flow cytometry, Serology, ELISA, Immunoassays, and culturing techniques are fundamental tools for both the hematologist and Immunologist. These tests not only aid in diagnosing anemia, certain cancers of the blood, inflammatory diseases, and certain bleeding and clotting disorders but are also important to understanding the underlying mechanism and pathologies of the immune system. These techniques are not only diagnostic

tools but also provide insights into the immune system's mechanisms and pathologies:

1. **Blood Tests:** Both hematologists and immunologists frequently use blood tests to assess various aspects of a patient's health. Common blood tests include:
 - a. **Complete Blood Count (CBC):** Provides information about the number and types of blood cells, including red blood cells, white blood cells, and platelets.
 - b. **White Blood Cell Differential:** Identifies different types of white blood cells (e.g., neutrophils, lymphocytes, eosinophils) and their proportions, which can provide clues about infections or immune system disorders.
 - c. **Blood Chemistry Panels:** Measure levels of electrolytes, proteins, enzymes, and other substances in the blood to assess organ function and detect metabolic abnormalities.
 - d. **Serology:** Serological tests detect antibodies or antigens in the blood and are commonly used in immunology to diagnose infections and autoimmune diseases.

2. **Techniques that may be used for blood tests or for other samples and experimental assays include the following:**
- a. **Microscopy:** Both fields may use microscopes to examine blood smears and other samples. Blood smears are especially important for hematologists to assess the morphology of blood cells.
 - b. **Flow Cytometry:** Flow cytometry is a powerful technique used in both fields to analyze and sort cells based on their surface markers and properties. It's valuable for characterizing different types of white blood cells, identifying abnormalities, and studying immune responses.
 - c. **Molecular Testing:** This includes various molecular techniques like polymerase chain reaction (PCR) and DNA sequencing. Hematologists might use molecular tests to diagnose genetic blood disorders, while immunologists use them to study the genetic basis of immune responses.
 - d. **Bone Marrow Biopsy:** Both fields may perform bone marrow biopsies to assess the production of blood cells and diagnose conditions like leukemia or myelodysplastic syndromes.
 - e. **Immunoassays:** These tests are widely used in both fields to detect and quantify specific proteins, hormones, antibodies, or other molecules in blood or other bodily fluids. More specifically, immunoassays are bioanalytical methods in which the quantitation of the analyte (ie protein, hormone, dna, etc) depends on the reaction of an antigen (analyte) and an antibody. A classic example of an immunoassay is:
 - f. **ELISA (Enzyme-Linked Immunosorbent Assay):** ELISA tests are used by immunologists to detect specific antibodies or antigens in blood samples, making them valuable for diagnosing infections or monitoring immune responses.
 - g. **Culturing Techniques:** Immunologists may use cell culture techniques to grow immune cells for various experiments. Hematologists may also culture cells to study blood cell disorders.

BACKGROUND

If immunology is the study of the immune system, which includes all the cells, tissues, and molecules that work together to protect the body from infections and diseases, then it is important to start familiarizing yourself with the cellular parts of the immune system.

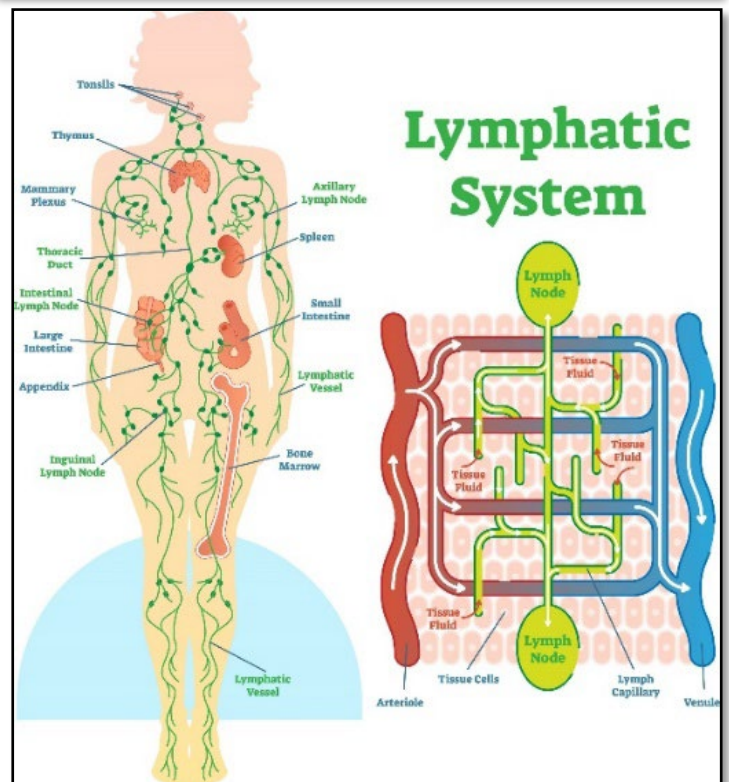
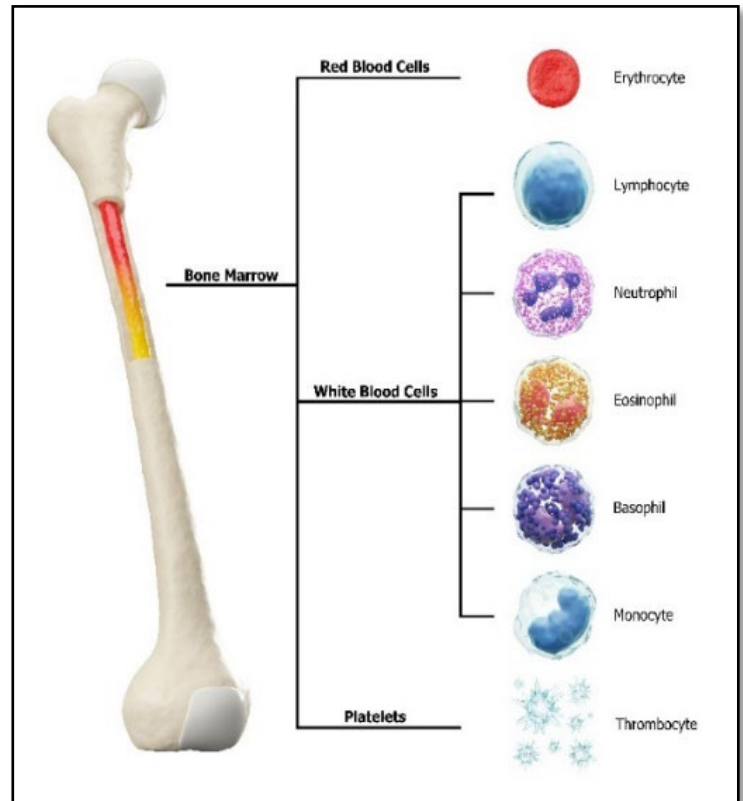
WHITE BLOOD CELLS (LEUKOCYTES)

White blood cells, or leukocytes, are a vital component of the blood, though less numerous than red blood cells (at a ratio of approximately 1:700). Leukocytes have nuclei and play a key role in protecting the body from infections. They consist of lymphocytes and monocytes, characterized by relatively clear cytoplasm, as well as three types of granulocytes with cytoplasm filled with granules.

LYMPHOCYTES

Lymphocytes originate in the bone marrow and mature in the thymus, and are similar in appearance under a microscope. They serve various functions, and although bone marrow is the ultimate source of lymphocytes, some that will become T cells migrate from the bone marrow to the **thymus**, where they mature. Both B cells and T cells also take up residence in lymph nodes, the spleen and other tissues where they encounter antigens; continue to divide by mitosis; mature into fully functional cells. The primary lymphocyte types are:

1. **B lymphocytes ("B cells")**: mature in the bone marrow, which is where they also acquire their antigen receptors (B cell receptors or BCRs). B cells play a central role in the adaptive immune response by recognizing antigens, producing antibodies.
2. **T lymphocytes ("T cells")**: This category includes subsets like:
 - a. **Cytotoxic T Cells (CD8+ T Cells)**: These are responsible for directly killing infected or cancerous cells
 - b. **Helper T Cells (CD4+ T Cells)**: These assist other immune cells, such as B cells and cytotoxic T cells, in their functions.
 - c. **Regulatory T Cells (Tregs)**: These help regulate the immune response and prevent it from becoming overly aggressive, avoiding autoimmune reactions.
 - d. **Memory T Cells**: These cells "remember" previously encountered antigens and provide long-term immunity.



MONOCYTES

Monocytes exit the bloodstream to become macrophages. These large, phagocytic cells engulf foreign materials (antigens) entering the body and dead or dying cells.

NEUTROPHILS

Neutrophils are the most abundant white blood cells. They migrate from capillaries into infected tissues, where they combat invaders, like bacteria, through phagocytosis. Neutrophils play a crucial role in preventing opportunistic infections, which can become life-threatening under conditions like radiation exposure or chemotherapy.

EOSINOPHILS

Eosinophils are typically low in number in the blood but increase significantly in diseases caused by parasitic worms. They release cytotoxic granules to combat invaders.

BASOPHILS

Basophils increase during infections and accumulate at sites of inflammation. They release mediators like histamine, serotonin, prostaglandins, and leukotrienes, which contribute to the inflammatory process and allergic responses.

PERIPHERAL BLOOD SMEARS

A peripheral blood smear is a **test where a drop of blood is spread on a slide and then stained to be examined under a microscope**. This allows doctors to look at the [morphology \(shape and appearance\)](#) and counts of red and white blood cells and platelets. The results can help diagnose various conditions, including [anemia](#), [leukemia](#), and [infections](#).

Here's a more detailed explanation:

What it is:

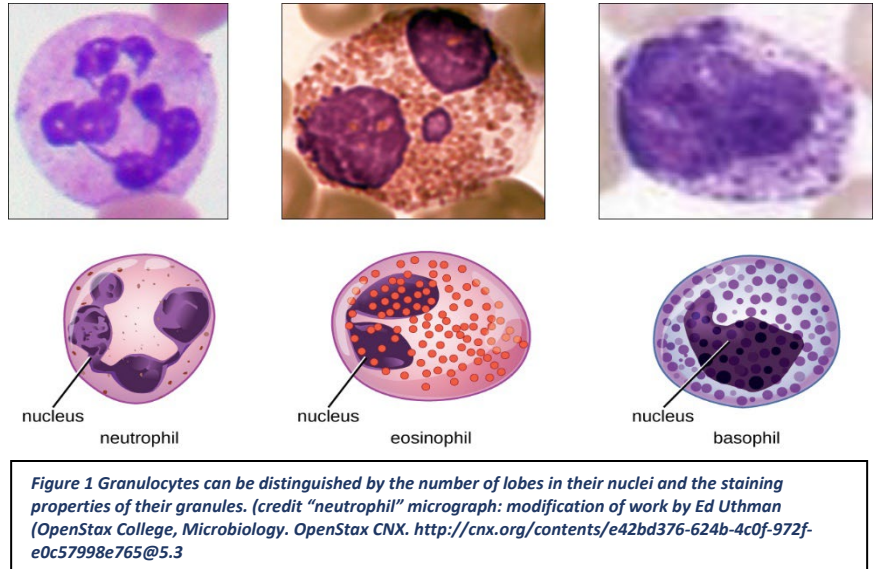
- A blood smear is a thin layer of blood spread on a glass slide and stained to make the cells visible under a microscope.
- The process involves collecting a blood sample, spreading it on a slide, staining it, and then examining it under a microscope.
- The smear allows doctors to see the individual blood cells and their characteristics, such as their size, shape, and internal structures.

Why it's done:

- To evaluate the different types of blood cells (red blood cells, white blood cells, and platelets).
- To help diagnose or monitor various conditions that affect blood cell populations.
- It can be used to detect blood parasites, like those causing [malaria](#) and [filariasis](#).
- It's often used to follow up abnormal results on a [complete blood count \(CBC\)](#).

What it can show:

- The number and type of red and white blood cells and platelets.
- Changes in the size, shape, and appearance of blood cells.
- The presence of parasites or other abnormal inclusions within the cells.



How it's interpreted:

- The results are interpreted by a hematologist or other healthcare professional trained in interpreting blood smears.
- Abnormalities in the blood cells can provide clues about underlying medical conditions.
- The results are often combined with other test results, a physical exam, and a medical history to make a diagnosis.

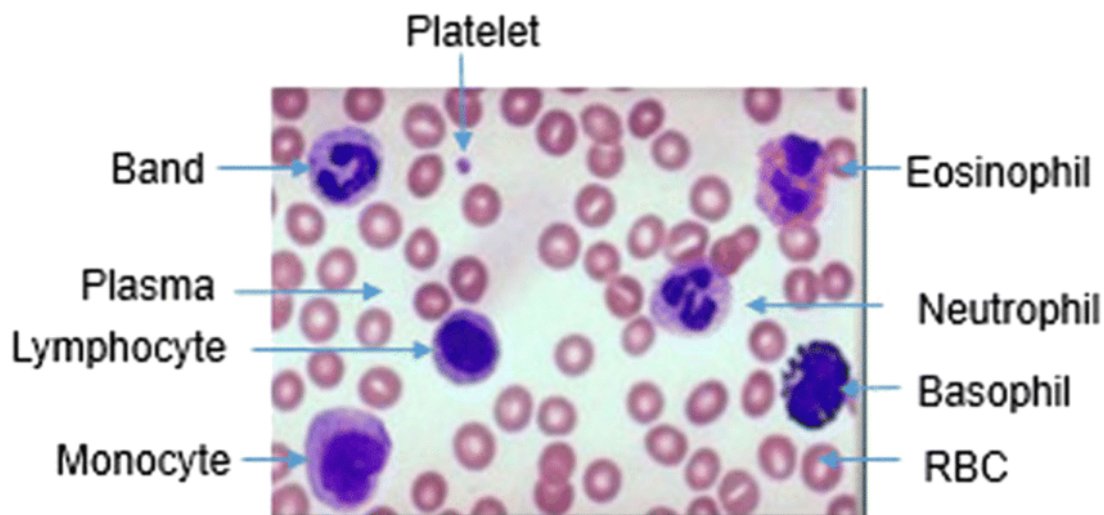
In summary, a peripheral blood smear is a valuable diagnostic tool that helps healthcare providers assess the health of blood cells and identify various conditions affecting the blood and bone marrow.

Microscopy:

1. Place the stained blood smear on the stage of a microscope.
2. Start with low magnification (10x or 20x) to locate areas of interest.
3. Switch to higher magnifications (40x or 100x) to examine different types of blood cells and assess their morphology.

Interpretation and Reporting:

1. Examine the blood cells for any abnormalities in size, shape, or number.
2. Count and classify different cell types, including RBCs, WBCs, and platelets.
3. Remember that blood smear preparation and interpretation require training and practice to ensure accurate results. It's a valuable tool for diagnosing and monitoring various medical conditions related to blood cells.

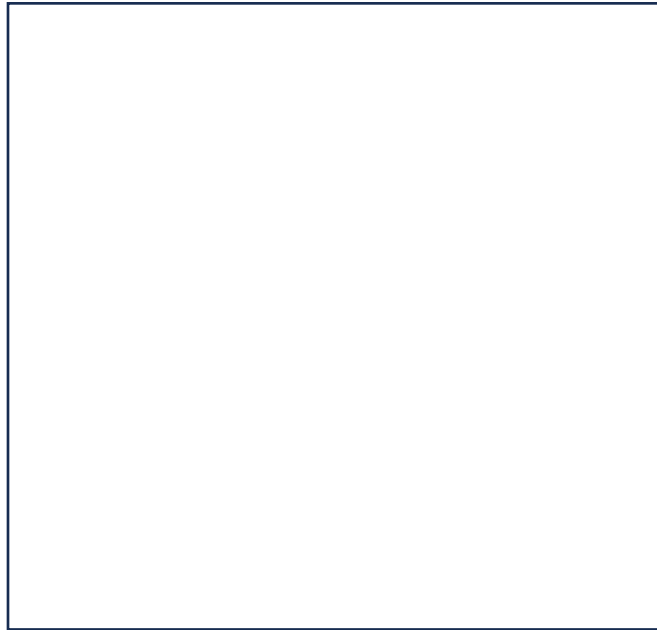


Blood Cells



LABORATORY GOALS

The lab activities over the next few weeks will cover a few of these techniques. Today's lab we will explore and practice counting and analyzing a **normal peripheral blood smear test**. Please draw here what you see on the blood smears.



NEXT WEEK –SEARCHING, READING, AND EXPLORING SCIENTIFIC LITERATURE AND WRITING A RESEARCH PROPOSAL

- Read the journal articles provided on Blackboard.
- Bring a computer or laptop to lab.

Brief Objectives for the lab

- Learn how to:
 - Use scientific databases
 - Cite literature
 - Understand different types of scientific literature

PART I SCIENTIFIC LITERATURE

Students will be able to:

- Characterize each section of a journal article, including the purpose of each section.
- Read, understand, and interpret scientific articles.
- List and understand the limitations of a given scientific study.
- Apply the results of a scientific study to make informed decisions
 - Describe and interpret key aspects of each paper
 - Explain the techniques used in this paper.
 - Summarize and interpret key results that supported the authors' conclusions.
 - Paraphrase the authors' main conclusions and evaluate how they contribute to our current understanding of Immunology

PART II WRITING A RESEARCH PROPOSAL

- List and describe the structure of a research proposal
- Analyze the elements of a research proposal
- Identify a relevant research topic

- Browse research literature to select a relevant research question.
- Pitch an idea to team members

LEARNING GOALS

- Science Process Skills:
 - Developing discernment and scientific literacy
 - Reviewing prior research
 - Interpreting results/analyzing data

LEARNING OBJECTIVES:

PART I SCIENTIFIC LITERATURE

Students will be able to:

- To effectively read, comprehend, and analyze scientific literature for research and academic purposes.
- Characterize each section of a journal article, including the purpose of each section.
- Read, understand, and interpret scientific articles.
- List and understand the limitations of a given scientific study.
- Apply the results of a scientific study to make informed decisions
 - Explain the techniques used in this paper.
 - Summarize and interpret key results that supported the authors' conclusions.
 - Paraphrase the authors' main conclusions and evaluate how they contribute to our current understanding

BACKGROUND: PART I READING SCIENTIFIC LITERATURE

Reading, comprehending, and learning from scientific literature is an iterative process that relies on several cycles of review, synthesis, critique and analysis. This will vary depending on the specific research area or topic you want to explore, how new you are to the field, or how much expertise you have built up. You will develop these skills the more practice you have, and it will require learning which academic databases, prompts are appropriate to use in your area of research interest and more importantly developing some sense of discernment regarding sources of research literature are most appropriate for your review.

Instructor-approved database search tools often include [NCBI/NLM](#) and [Google Scholar](#). It's essential, however, to consider additional factors when evaluating the quality and suitability of research articles. This includes checking whether articles are recent and peer-reviewed to ensure credibility. Furthermore, one should assess the reputation of the journal and the transparency of its editorial processes. Seeking input from colleagues can also provide valuable insights into the quality and impact of research papers, extending beyond their mere publication. You can also find out *chisme* (the tea, gossip) or important insights to how work is done in the lead researcher's lab, and the quality and success of students graduating from their labs. Questions like "Who are they?" "What is the journal's [Impact Factor](#)?", "Where are they located?" "What is their publication track record?" and "How many citations do their works receive?" become valuable in understanding their influence and accountability within the scientific community.

As a general guideline, many researchers tend to favor journals affiliated with respected academic institutions or professional societies. However, it's important to note that valuable research gems can emerge from lesser-known sources. Dismissing a paper solely based on the institution's "supposed" reputation would be unwise. In your scientific journey, you may encounter outstanding work from less prominent institutions that are home to outstanding researchers that have established themselves through the quality of their scientific work.

The ability to read scientific literature effectively not only enhances your scientific literacy but also sharpens your discernment. As you progress in your scientific endeavors, you'll develop a refined sense of what constitutes valuable knowledge, what questions you can confidently address, and which areas require further exploration.

Welcome to your formal training as a scientist where scientific literacy and discernment are valuable guides, and knowledge is the ultimate destination.

ACTIVITIES

If reading a scientific research paper was like going to the gym, this paper would be like your first day of CrossFit. Don't worry, we'll go slowly. I highly recommend you do the pre-lab prep activity as a warm-up for the in-lab activity. Locate the following paper we will be reviewing in lab this week; use Google Scholar or PubMed to locate the journal article and download the PDF:

- **Bararia D, Kwok HS, Welner RS, Numata A et al. Acetylation of C/EBP α inhibits its granulopoietic function. *Nat Commun* 2016 Mar 23;7:10968. PMID: 27005833 PMCID: PMC4814574 DOI: 10.1038/ncomms10968.**

I'll bring the printed copy of the paper for the lab activity, if you want to save on printer ink.

PRE-LAB ACTIVITY WARM-UP

1. Skim the Article:

- Read the title, abstract, and keywords to grasp the article's main focus.
- Identify the authors, affiliations, & any conflicts of interest. What is the impact factor of the journal?
- Look for key terms, concepts, or hypotheses that stand out.

2. Assess Article Structure:

- Familiarize yourself with the typical sections of a scientific article: Introduction, Methods, Results, Discussion, and Conclusion (IMRAD).
- Pay attention to any additional sections like Materials and Methods, References, or Supplementary Information.
- Note the headings and subheadings within each section.

3. Begin In-Depth Reading:

- Start with the Introduction:
 - Identify the research question or problem.
 - Understand the rationale for the study.

Note: if you are feeling motivated, go ahead and try to do the following steps for the in-class part.

STOP

LABORATORY 4 (IN PERSON): LITERATURE REVIEW & DISEASED PERIPHERAL BLOOD SMEARS

IN CLASS GROUP ACTIVITY

4. Continue In-Depth Reading:

- Review and share your findings from the PRE-LAB activity warm-up.
- Move to the Methods:
 - Examine how the study was conducted.
 - Assess the experimental design and data collection methods.
- Analyze the Results:
 - Interpret the figures, tables, and graphs.
 - Identify significant findings and trends.
- Review the Discussion:
 - Understand the authors' interpretation of the results.
 - Consider how the study contributes to the field.
- Conclude with the Conclusion:
 - Summarize the key takeaways.
 - Reflect on the implications of the research.

5. Discuss with your group and Take Notes:

- a. While reading, jot down important points, questions, and any unclear concepts.
- b. Highlight key parts for future reference.

6. Consult References:

- a. Check the references for articles for further reading.
- b. Verify the credibility of the cited works.

7. Analyze the Data:

- a. Evaluate the quality & relevance of the data presented.
- b. Consider the statistical methods used for data analysis.

8. Critical Thinking:

- a. Formulate questions about the article's strengths and weaknesses.
- b. Assess the validity of the study's conclusions.
- c. Compare the article with other sources on the same topic.
- d. Reflect on how the article contributes to your research or understanding of the topic.
- e. Write a concise summary of the article, highlighting its main points.

9. Summarize and Reflect:

- a. If you encounter unfamiliar terms or concepts, consult textbooks or reputable sources to gain a better understanding.

CLASS DISCUSSION

10. Instructor Guided Discuss and Share:

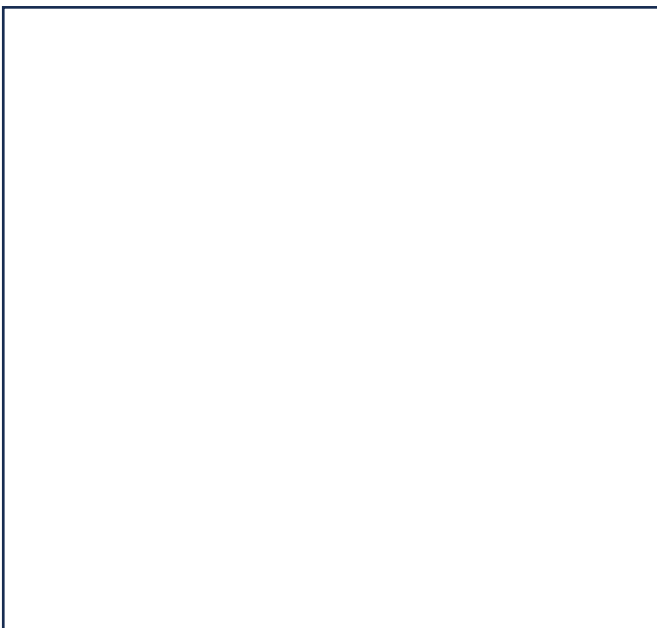
- a. Engage in discussions with peers to gain different perspectives.
- b. Share your insights.

In what ways will understanding this research paper help you with your research proposal?

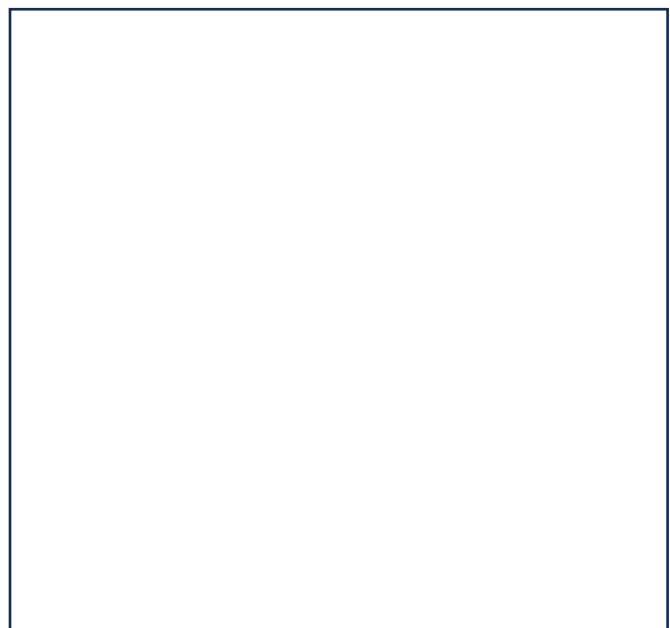
Remember that understanding scientific literature is a skill that improves with practice. Over time, you'll become more adept at extracting valuable information from research articles to support your academic or research endeavors.

DRAW OUT THE DIFFERENCE BETWEEN A NORMAL AND DISEASED PERIPHERAL BLOOD SMEAR

Normal



Diseased



CELLULAR TECHNIQUES

Cell culturing is a fundamental tool within the life sciences, playing a critical role in answering basic scientific and translational research questions across disciplines. Maintaining healthy cultures is vital to obtaining reliable, high-quality data. Here, we explore considerations and troubleshooting tips from cryopreservation to tissue culture.

Cell culturing is hard work. Because it's a highly technical process, there is a lot that can go wrong when you take cells from one source and manipulate them in another, from contamination to lackluster *in vitro* growth.

Cell Seeding from a Cryogenic State

In buying cell culture lines, products come cryopreserved, which is necessary to maintain cell viability long-term. From that state, you'll need to thaw out and seed the cells, which shouldn't be rushed, even though it happens fast. This important step often does get hurried and sometimes overlooked, which risks contamination and doesn't give the cells the good, sterile start that they need.

Here is the correct way to conduct the cell seeding process:

- 1) Get all of your supplies ready. You'll need your cryopreserved vial, a beaker or water bath filled with pre-warmed water, and a flask with pre-equilibrated media. You'll also need a conical vial filled with media if you intend to spin down the cells and remove the DMSO or other cryoprotectant.
- 2) Warm the vial. Gently swirl the cryopreserved vial in the beaker of pre-warmed water until the contents are almost completely thawed. Then, clean the vial with an alcohol wipe to reduce contamination risk.
- 3) Seed your cells. Using a pipette, remove the cells from the cryovial and transfer them to the flask. Then, pipette the flask contents up and down to mix the solution for easy seeding. With that, your cells are thawed, transferred, and ready for DMSO removal.
- 4) Remove the DMSO. Centrifuge the cells at 1200rpm for 5 minutes and decant to remove residual cryoprotectant (e.g. DMSO). Resuspend the cells in fresh media and move to the incubation flask.
- 5) Culture the cells. Place your flask in a 37C incubator with 5% CO₂ for recovery upon thawing.

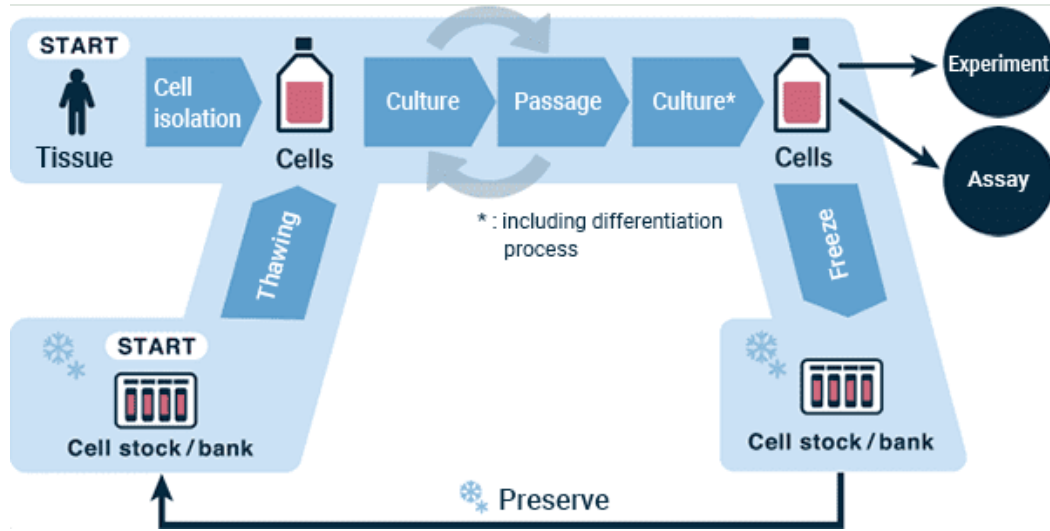
Harvesting Your Suspension Cells

- 1) Remove the media and cells from the flask and place in a 15mL or 50mL Falcon tube.
- 2) Centrifuge the cells from 1200rpm in a tabletop centrifuge for 5 min. Decant the supernatant.
- 3) Wash cells 1X with warm PBS.
- 4) Harvest cells for downstream applications like RNA, DNA, or protein extraction by transferring them to an Eppendorf tube and centrifuging one more time at 1200rpm for 5 minutes. Decant the supernatant.
- 5) Snap freeze the cell pellets and store at -80C.

Making Cell Culture Work for You

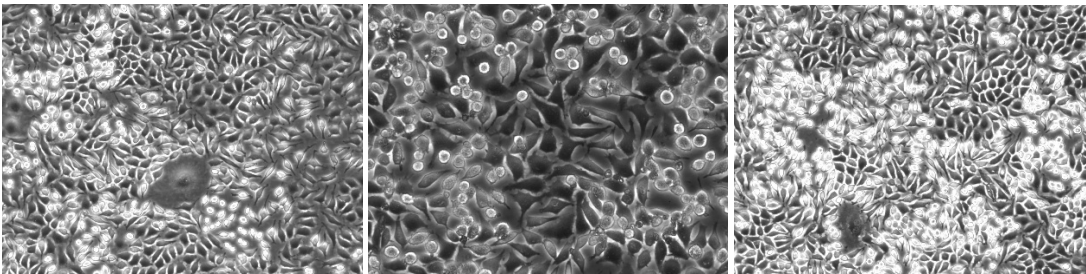
Cell culture can open up your lab to a whole new world of experimentation and downstream applications – it can be fun, too! But there can be a lot at stake when you're manipulating live cells, so it's important to do so correctly. By following

these essential tips and working your way up to more advanced protocols, you too can become a cell culture pro in no time!



CULTURE OF L929 AND WEHI CELLS

L929 CELLS



L-929 cells are a fibroblast-like cell line derived from the subcutaneous connective tissue of a 100-day-old male C3H/An mouse. Established in the 1940s, this cell line has become pivotal in various biological and medical research fields due to its robustness, ease of culture, and versatility in applications.

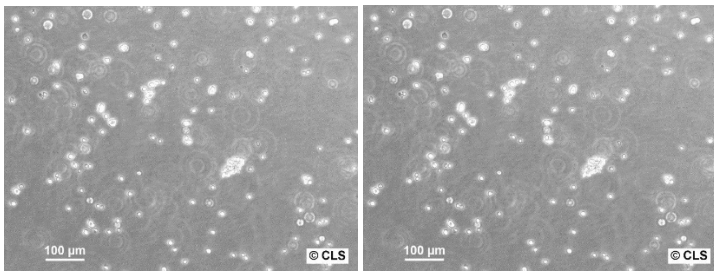
L-929 cells are characterized by their spindle-shaped, fibroblastic morphology, and adherent growth. They are widely used in cytotoxicity assays and serve as a standard model to assess the biocompatibility of materials and the toxic effects of various substances, which is particularly relevant in the fields of biomaterials and tissue engineering.

L-929 cells are also employed in the study of cytokine activity, especially in assays for necrosis factor (TNF) activity, due to their sensitivity to TNF-induced cytotoxicity. This makes them valuable in immunology and inflammation research.

L-929 cells are further utilized in virology as a host for viral replication studies. Their susceptibility to various viruses, such as the infectious bursal disease virus (IBDV), facilitates the investigation of viral life cycles, host-virus interactions, and the efficacy of antiviral compounds.

Overall, the L-929 cell line is a valuable resource in scientific research and offers a versatile platform for studies in cytotoxicity, immunology, virology, and biomaterials.

WEHI CELLS



The WEHI-3 cell line is a murine leukemia cell line, specifically derived from the BALB/c strain. It was originally established from a spontaneous myelomonocytic leukemia found in a mouse. This cell line is extensively used as a model to study myeloid differentiation and the immune response, particularly the mechanisms underlying leukemia progression and the response of leukemic cells to various treatments. WEHI-3 cells are capable of producing interleukin-3 (IL-3) and are often used in research as a source of this cytokine.

In laboratory settings, WEHI-3 cells have been employed to assess the differentiation potential of various compounds and the biological activities that modulate the hematopoietic system. These cells have proven instrumental in understanding how alterations in gene expression affect myeloid cells, serving as a critical tool in the development of therapeutic strategies against myeloid leukemias. The cell line is also used *in vivo* to establish murine models of disease through transplantation into susceptible mouse strains, enabling studies of tumor progression and the efficacy of anti-cancer agents.

LABORATORY 6 (IN PERSON): ELISA – M-CSF AND/OR IL-3

ANTIBODIES AND ELISA

Background

What is an ELISA?

An ELISA is a type of immune-assay. Immuno assays are a group of powerful techniques that have been developed recently. Using the body's own defense system, scientists can determine the quantity of very small amounts of specific molecules. The tests are quick and yield information that would be hard to obtain using other methods. ELISA is an Enzyme Linked Immuno Sorbent Assay. It is used to determine the level of antibodies in a sample. These tests are useful because they are so specific and are relatively simple to perform. Using an [enzyme](#) linked to the antibody, scientists can accurately measure the quantity of antibody, and therefore antigen, present.

What can it be Used For?

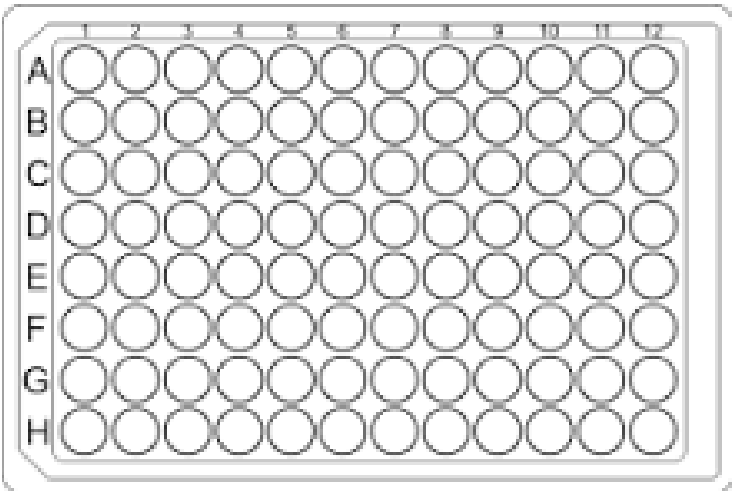
It is used to identify the type of [antigen](#) and the quantity that is present in a sample.

Antibodies can be used to detect the presence of drugs, [viral](#) and [bacterial](#) products and other unusual or abnormal substances in the blood. This is important because of the role antibodies play in disease. For example, antibodies can be used to develop a vaccine for a specific disease and to diagnose that disease.

Mammals make antibodies to almost anything that is not normally part of the body such as viruses and bacteria. You can get a mammal to make an antibody to almost anything by injecting it into the bloodstream.

[Antibodies](#) are really useful because they are so specific. They latch on to only one type of antigen and ignore all the others. Hence it is easy to identify any antigens that are present in the sample.

An HIV ELISA, sometimes called an HIV enzyme immunoassay (EIA) is the first and most basic test to determine if an individual is positive for a selected pathogen, such as HIV. The test is performed in a 8 cm x 12 cm plastic plate which contains an 8 x 12 matrix of 96 wells, each of which are about 1 cm high and 0.7 cm in diameter. The next page illustrates how an HIV ELISA is performed.



An ELISA plate

Learn more about [R&D Systems Quantikine® QuickKit™ ELISAs](#)

QuickKit Important Notes

There are a few important things to keep in mind before we begin.

- First, some proteins are detectable in saliva, so it may be important to wear a facemask to prevent contamination. Refer to the precautions section in your kit booklet for specifics.
- Also, be sure to wear personal protective equipment and refer to the Safety Data Sheet on our website prior to use.
- Finally, protocols vary by kit and sample, so read your kit booklet carefully before you begin and during each step for specific instructions.

This kit is validated for cell culture supernates, serum and plasma samples. We recommend that all samples are assayed immediately after they are collected, but samples can be frozen for future use.

Plate Setup

1. If you haven't done so already, take some time to think about how you'll setup your plate. In our plate the standard curve will be setup in duplicate in strips one and two, and our controls and samples in duplicate in strips three and four. This is also where you can consider running additional dilutions of your samples.
2. Follow the recommended sample dilution factor in the sample preparation section of your kit booklet. If a recommended dilution is not listed, samples can be run neat. Multiple dilutions are recommended for unknown samples. Keep in mind that many cytokines, such as TNF-alpha shown in this video, are expressed at very low abundance in serum and plasma from healthy individuals and will likely fall below the standard curve.

Wash Buffer Preparation

3. To prepare reagents, first bring all kit reagents to room temperature. To prepare your wash buffer, add 10 mL of wash buffer concentrate to 240 mL of deionized or distilled water in a graduated cylinder to yield 250 mL of wash buffer. Mix gently.
4. Next, prepare your antibody cocktail. Reconstitute the Human TNF-alpha Capture Antibody Concentrate with 400 uL of the specified diluent. Gently mix to ensure complete reconstitution. Avoid vigorous mixing and let rest on benchtop for a minimum of 5 minutes.
5. Next, in a 15 mL conical tube, add 300 uL of the reconstituted capture antibody concentrate and 300 uL of the detection antibody concentrate to 5.4 mL of the specified diluent. This produces 6 mL of Antibody Cocktail. Avoid vigorous mixing.

Standard Preparation

6. Next, prepare your Human TNF-alpha Standard. Refer to the vial label for specific reconstitution volume. Reconstitute according to the kit booklet. This reconstitution produces a stock solution of 20 ng/mL. Gently mix the standard to ensure complete reconstitution and let it sit for a minimum of 15 minutes on the benchtop prior to making dilutions.

Standard Curve Preparation

It's time to create the standard curve.

7. Pipette 50 uL of the standard into the 2000 pg/mL tube. Then, add 450 uL of calibrator diluent into the same tube. This creates the high standard. Vortex gently to mix.
8. Check your kit booklet to ensure you have the right volume and diluent. Next, pipette 200 uL of the appropriate calibrator diluent into the remaining tubes. Use the high standard to produce a 7-point standard curve. Be sure to mix each tube thoroughly by very briefly vortexing. If you don't have a vortexer you can lightly tap the side of the vial or pipette up and down. Try to minimize foaming and bubbles.
9. Change pipette tips before transferring to the next tube. The calibrator diluent serves as the blank. Blank wells contain zero standard and are treated identically to assay wells. They serve as the non-specific binding control for all the assay reagents.

Preparing Controls

10. In this assay, we're using the kit-specific [R&D Systems QC Controls, Catalog number QC259](#). Check your kit booklet for the analyte-specific controls. These should be reconstituted according to their lot-specific certificate of analysis and analyzed as-is, without dilution. If you're not using [R&D Systems QC Controls](#), we recommend formulating your own control.

Loading the Plate

11. Once you've prepared your samples and reagents as directed in the previous sections, it's time to run your QuickKit ELISA. We recommend that all samples, controls, and standards be assayed in duplicate. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal. We recommend labeling the plate strips.
12. First, add 50 uL of standard, control, or sample to each well. Then add 50 uL of Antibody Cocktail per well. It is important to follow this order and use consistent pipetting technique. Avoid contact with pipette tips and the solution in the well when adding the antibody cocktail. When all samples and solutions have been added to the plate, cover the plate with the adhesive strip provided and ensure it is completely sealed.
13. Incubate the plate for 1 hour at room temperature on the shaker at a point-one-two-inch orbit at 500 plus-or-minus 50 rpm.

14. In the last 5 minutes of your incubation, prepare your substrate solution. Mix together color reagents A and B in equal volumes. This substrate solution must be protected from light and used within 15 minutes. It should remain colorless until added to the plate. 100 μL of the mixture is required for each well.

Wash Step

15. Next, Aspirate each well and wash by filling each well with 400 μL of [Wash Buffer](#) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. Washing with a multi-channel pipette is too gentle and is not recommended. We recommend adding a 30 second soak period after adding wash buffer. Wash for a total of three times and after the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

Assay Procedure

16. Next, add 100 μL of Substrate Solution to each well. Remember to protect the substrate solution from light.
17. Now incubate for 20 minutes at room temperature on the benchtop.
18. Next add 50 μL of Stop Solution to each well. Stop solution should be added to the plate in the same order as the substrate solution. It is important to quickly dispense the stop solution into the well at a 45-degree angle so the color in the wells changes completely from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing or place the plate back on the shaker. As a last resort, you can use a pipette tip to individually mix each well, but this may result in loss of volume and could impact your results.

Collecting Results

19. Now you can collect your results. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. To evaluate your data, the average of the blank wells should first be subtracted from all wells. If wavelength correction is available, set to 540 or 570 nm. This will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate due to the slight imperfections in the plastic of the 96-well ELISA plate. Plot your standard curve and read controls and unknowns off the standard curve. The blank wells should not be incorporated into the standard curve.

This concludes our video guide for running an [R&D Systems QuickKit ELISA](#). For more helpful protocols subscribe to our YouTube channel. For more information about [R&D Systems QuickKit ELISAs](#) visit [R&D Systems.com](#) or contact us.

All ELISA Kits and Services

- [More information about ELISA kits](#)
- [View all ELISA Kits](#)
- [More about Quantikine ELISA Kits](#)
- [Custom Assay Services](#)

Additional ELISA Kit Resources

- [View all ELISA protocols](#): Troubleshooting guides, protocols and more.
- [ELISA Guide](#): Get a full overview of R&D Systems ELISA kits
- [ELISA FAQs](#): Read the most commonly asked questions about our ELISA kits.

[ELISA Protocols](#)

Relevant Protocols from R&D Systems

***Analysis of results:** An ELISA can give qualitative (yes or no) or quantitative (how much?) information. Qualitative results can be determined visually by eye. For quantitative determination of precise concentrations, a microplate reader is required.

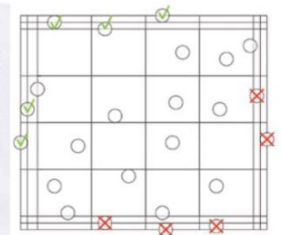
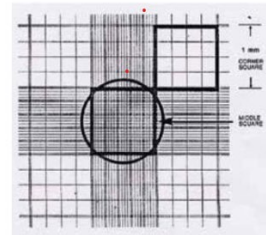
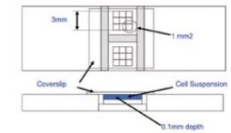
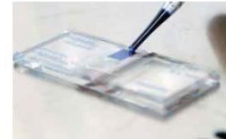
LABORATORY 7 (IN PERSON): CULTURE OF 32DCL3 CELLS, COUNTING, AND ADDITION OF CYTOKINES

OVERVIEW OF PROCEDURES

In many laboratory applications, determining cell concentration is necessary. Spectrophotometry can assess cell density but doesn't provide information on cell viability or types. A counting chamber, such as a **hemocytometer**, is designed for to determine cell concentration and in some applications allows you to determine concentration of red blood cells and white blood cells differentially.

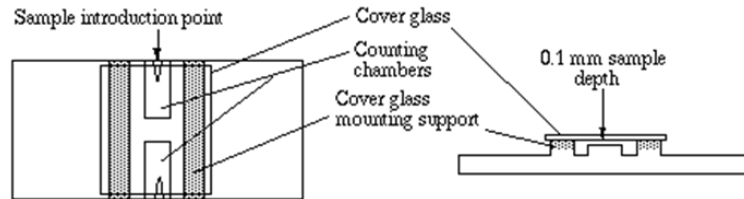
KEY POINTS FOR TODAY'S EXPERIMENTS: USING A HEMOCYTOTR

1. Trypan Blue is toxic and is a potential carcinogen. Protective clothing, gloves and face/eye protection should be worn. Do not breathe the vapor.
2. The central area of the counting chamber is 1 mm^2 . This area is subdivided into 25 smaller squares ($1/25 \text{ mm}^2$). Each of these is surrounded by triple lines and is then further divided into 16 ($1/400 \text{ mm}^2$). The depth of the chamber is 0.1 mm .
3. The correction factor of 10^4 converts 0.1 mm^3 to 1 mL ($0.1 \text{ mm}^3 = 1 \text{ mm}^2 \times 0.1 \text{ mm}$)
4. There are several sources of inaccuracy:
 - a. The presence of air bubbles and debris in the chamber
 - b. Overfilling the chamber such that sample runs into the channels or the other chamber
 - c. Incomplete filling of the chamber Cells not evenly distributed throughout the chamber
 - d. Too few cells to count. This can be overcome by centrifuging the cells, re-suspending in a smaller volume and recounting
 - e. Too many cells to count. This can be overcome by using a higher dilution factor in trypan blue or PBS e.g. 1:10
 - f. To prepare the counting chamber, clean its polished surface and the coverslip. Coverslips for counting chambers are thicker to overcome liquid surface tension. Fill one of the V-shaped wells with your cell suspension, ensuring the mirrored surface is just covered. Then, place the charged chamber on a microscope stage and focus on the counting grid at low power. Use caution with higher power objectives due to the chamber's thickness.
 - g. To perform the count, determine the needed magnification, and systematically count cells in selected squares to reach a statistically significant count (usually 100 cells). For accurate results, establish a specific counting pattern and account for overlapping cells.
 - h. As 10X objective lens is correct for white blood cells counting, count correctly the entire no. of cells present in 4 big corner squares.
 - i. To count the red blood cells, the microscope essential be transferred to a 40X objective lens. Count the cells in the 4 separate zones as identified initially.
 - j. All cells which are counted, write down on clean paper for calculation.
 - k. If cells are touching/stirring the 4 perimeter/border sides of a corner square, only counted cells on 2 borders, whichever the 2 external margins or 2 internal borders



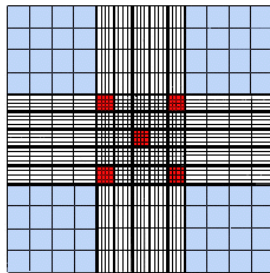
EXPERIMENTAL PROCEDURE:

1. Clean the Neubauer chamber and the cover slip with 70% ETHANOL. Put the glass cover on the Neubauer chamber central area. Use a flat surface to place the chamber, like a table or a workbench.



2. Charge the Hemocytometer - If the sample has been allowed to stand, mix the diluted blood thoroughly to resuspend the cells.
3. Using PBS dilute the blood 1:1000 and 1:10000
4. With a micropipette take 20 μL of the diluted blood and carefully put it in the hemocytometer, by inclining the micropipette in an angle of approx. 30° just next to the chamber cover slip.
5. Allow the cells to settle for a few minutes to ensure they are evenly distributed across the grid.
6. Count and Calculate - An erythrocyte count is done with a Neubauer hemocytometer as follows:
7. Place the hemocytometer on the stage of a microscope and start with a low-power objective (e.g., 10x or 20x).
8. Focus on the grid lines and begin counting RBCs within specific grid squares.

■ areas of the grid where WBC are counted



■ areas of the grid where RBC are counted

9. Using 40X magnification, Of the 25 medium squares, only the four corner squares and the center square within the large center square are used to perform RBC counts.
 - a) Each of the nine squares in the Improved Neubauer grid has a volume of 0.1 mm^3 . The multiplication factor of 10^4 in the formula above converts the count from cells per 0.1 mm^3 to cells per ml. Most hemocytometer squares have a volume of 0.1 mm^3 , so the multiplication factor will be 10^4 in most cases.
 - b) Out of these 25 squares, the RBCs are counted in 5 squares. So the Area of 5 small squares is 5/25 i.e. 1/5
 - c) Total RBC Count = $N \times \text{Dilution} / \text{Area} \times \text{Depth}$
 - i. Since the volume of 1 big square is: 0.1 mm x 0.1 mm = 0.01 mm^2 of area counted.
 - ii. Since the depth of the chamber is 0.1mm; 0.1 mm = 0.01 cm
 - iii. (area x depth) = volume; 0.01 $\text{mm}^2 \times 0.01 \text{ mm} = 0.0001 \text{ mm}^3 = 0.0001 \text{ ml} = 0.1 \mu\text{L}$ or .0001 ml
 - iv. Total RBC count = $N \times 10,000 / \text{mm}^3$
10. Limitation of the Procedure - A highly elevated erythrocyte count may make accurate counting difficult. In this instance, a secondary dilution should be made. When calculating the total count, adjust the formula to allow for the secondary dilution.
11. Technical Note - Cells and diluent(s) must be adequately mixed and counting chambers should be properly filled if errors in manual counting procedures are to be avoided.
12. Use the formula to calculate the RBC concentration per microliter (μL) or milliliter (mL) of the original blood sample:

Protocol:

A. Culture and processing of mouse myeloid precursor cells

Keep on ice

B. Cell counting with hemocytometer

- 1) Pipette 90 μL 0.4% trypan blue into an Eppendorf tube.
- 2) Pipette 10 μL cell suspension from your first sample into well with trypan blue.
- 3) Mix well and pipette 10 μL onto hemocytometer.
- 4) Use microscope to count cells on hemocytometer. You want to count the 16 boxes that make up the corners of the hemocytometer. Average the counts of two 16 box sections of the hemocytometer for your count. Write down the number below in step 6.
- 5) Repeat steps 1-4 for each remaining sample you are counting.
- 6) Calculate your counts by multiplying first by 10^4 and then by the dilution factor (which is 10 in this case) and how many mL of cell suspension you have (see equation):

32Dcl3 #1 +IL-3: _____ cells $\times 10^4 \times 10$ (d.f.: 10 μL in 100 μL final vol.) = _____ cells/mL

Once you have your counts, move to Part C.

C. Diluting 32Dcl3 cells in RPMI w/cytokines

- 1) Calculate the dilution of 32Dcl3 cells to 1.5×10^5 cells/mL in 10 mL of RPMI culture medium (**$C_1 \cdot V_1 = C_2 \cdot V_2$**)
- 2) Prepare 4 flasks of 32Dcl3 cells with 10mL of cells
 - a. Add 1mL 10% WEHI (IL-3) conditioned medium to flask #1 (be sure to label the flask)
 - b. Add 5 μL of granulocyte-colony-stimulating factor (G-CSF) to flask #2 (be sure to label the flask)
 - c. Add 5 μL of monocyte-colony-stimulating factor (M-CSF) to flask #3 (be sure to label the flask)
 - c. Add no cytokines to flask #4 (be sure to label the flask)

D. Incubate the resulting cells

- c. Place cells in the incubator at 37C with 5% CO_2
- d. Incubate cells for 7 days

LABORATORY 8 (IN PERSON): HARVEST OF 32DCL3 CELLS FOR MULTIPLE DOWNSTREAM ASSAYS

HARVESTING CELLS FOR RNA EXTRACTION

Protocol:

A. Culture and processing of mouse myeloid precursor cells

Keep on ice

B. Harvest cells for downstream experiments

- 1) Harvest the 32Dcl3 flasks into 3 separate 15mL Falcon tubes.
- 2) Centrifuge the tubes at 1200rpm for 5 min. Decant supernatant.
- 3) Wash the cells once in 10mL 1X PBS.
- 4) Centrifuge the tubes at 1200rpm for 5 min. Decant supernatant
- 5) Resuspend the cells in 1mL 1X PBS
- 7) Centrifuge the tube at 5000rpm for 5 min. Decant supernatant
- 8) Resuspend the pellet in RNA extraction buffer and store at -20C.

CYTOSPINS FOR MICROSCOPY

- 12) Transfer 500 μ L of the washed cells to an Eppendorf tube for cytopspin and microscopy.
- 13) Walk to the Eiring laboratory to perform cytopspins with the resulting cells. Be sure to label your slides appropriately (e.g. No Cytokines, IL-3, G-CSF, GM-CSF)
- 14) Fix the resulting slides with methanol for 2 minutes, allow to air dry.
- 15) Store the slides at room temperature until the following week.

LABORATORY 9 (IN PERSON): WRIGHT-GEIMSA STAINING AND MICROSCOPY

WRIGHT-GEIMSA STAINING

Wright-Giemsa staining is a widely used technique in hematology and cytology for differentiating cells in blood smears and bone marrow aspirates. It's a type of Romanowsky stain that utilizes a combination of **methylene blue** (basic dye, stains blue or purple) and **eosin** (acidic dye, stains red or orange). This staining method allows for clear visualization and differentiation of cellular components, making it valuable for diagnosing various conditions like leukemia, anemia, and infections.

Here's a more detailed explanation:

What it is:

Wright-Giemsa stain is a mixture of Wright's stain and Giemsa stain.

Wright's stain is a Romanowsky stain composed of eosin and methylene blue.

Giemsa stain contains methylene blue, azure, and eosin.

This combination allows for differential staining of cells, highlighting different cellular components based on their chemical properties.

How it works:

Basic components (like DNA in the nucleus) stain blue or purple: because they are attracted to the acidic dye eosin.

Acidic components (like hemoglobin in red blood cells) stain red or orange: because they are attracted to the basic dye methylene blue.

Applications:

Blood smears: Used to identify and differentiate various white blood cell types, red blood cell morphology, and assess platelet numbers.

Bone marrow aspirates: Used to evaluate the cellular composition of bone marrow, aiding in the diagnosis of hematological disorders.

Detection of parasites: Wright-Giemsa stain can be used to identify parasites in blood, such as malaria parasites (*Plasmodium* species).

Cytological studies: Used to examine cells from various body fluids (e.g., cerebrospinal fluid, pleural fluid) to identify abnormal cells.

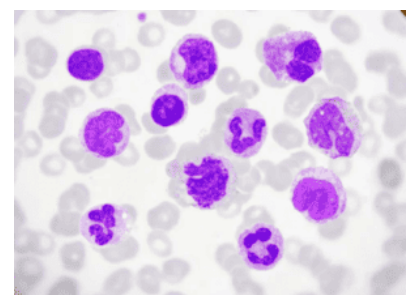
Procedure:

Prepare the smear: A thin smear of blood or other sample is made on a clean glass slide.

Staining: The slide is flooded with Wright-Giemsa stain, then diluted with a buffer or distilled water.

Rinsing and drying: The slide is rinsed with water or buffer and air-dried.

Microscopic examination: The stained smear is examined under a microscope, typically at high magnification, to visualize and analyze the cells.



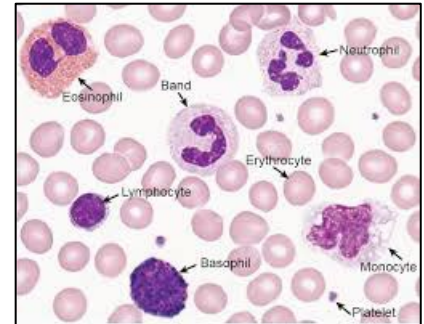
Protocol:

- **Prepare the Wright-Giemsa Working Solution:**

In a glass staining dish, prepare a 1:20 dilution by mixing 7.5 mL of Wright-Giemsa stock dye with **150 mL of double-distilled water (ddH₂O)**. Gently mix by pipetting up and down until the solution appears uniform in color.

- Place your slides into a slide rack and **slowly submerge them** in the Wright-Giemsa working solution, ensuring each slide is **fully covered**. **Incubate for 15 minutes** at room temperature.
- In a separate staining dish, fill with **double-distilled water (ddH₂O)**. After the 15-minute incubation, **quickly transfer the slide rack** into the rinse dish and **submerge for at least 5 seconds** to remove excess stain.
- Allow the slides to **air dry completely**. Once dry, examine the stained cells under a microscope. A properly stained slide should show **purple nuclei** and **pink cytoplasm**.

NOTE: REFER TO THE IMAGE FOR AN EXAMPLE OF A GOOD WRIGHT-GIEMSA STAIN.



Note:

Staining Troubleshooting:

Please note that **some slides may require further adjustments** to achieve optimal staining. If the stain appears too light or uneven, you may need to **extend the staining time** or **adjust the wash duration**.

- **Light staining** may benefit from a longer incubation in the Wright-Giemsa solution.
- **Overstaining or background staining** may be improved by increasing the rinse time.

Optimization may vary depending on **cell density, slide dryness, or reagent quality**; therefore, it's essential to adjust as needed for optimal results.

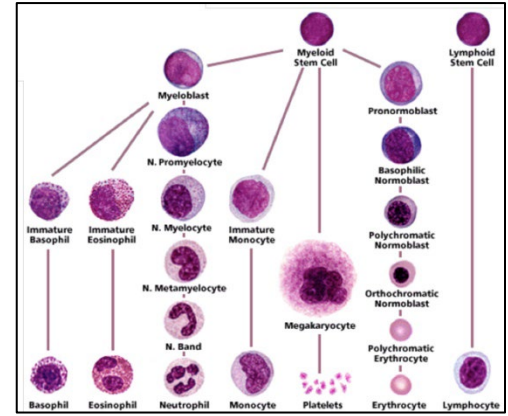
BRIGHTFIELD MICROSCOPY

- Imaging can be performed using an inverted light microscope with at least 60X magnification and a camera. However, for higher-quality images, we recommend using the LEICA LMD7000 Laser Microdissection Microscope available at the core facility.
To reserve time on the LEICA LMD7000 microscope, use the following link:
<https://www.utep.edu/science/csi/reservations.html>
- If your microscope or camera settings include an auto white balance or auto white background mode, enabling it can help produce more transparent backgrounds and improve overall image quality. Take images from several different areas of the slide to ensure you are capturing a representative sampling of the cell population. This helps avoid bias from localized variations in cell density or staining.

Quantifying Neutrophils on Wright-Giemsa-Stained Slides

1. Use the 60x dry objective to view the stained slide; this allows for high magnification without the need for immersion oil.
2. Adjust the light and focus to distinguish between nuclear and cytoplasmic features clearly.
3. Choose a representative area (avoid areas with overlapping cells or uneven stain)
4. Scan the slide in a systematic serpentine (Zigzag) pattern to avoid double-counting.

- While performing the differential count, use a **manual tally counter**. To keep track of specific cell types.
 - Use **one side** of the counter to record the number of **neutrophils**.
 - Use the **other side** to count the number of **blasts** or any other cell type of interest.
- Both neutrophil and blast counts should be recorded in the lab notebook to document the progress of differentiation and track the proportion of undifferentiated cells.



How do I know it's a neutrophil?

Nucleus

- Segmented or multilobed** (typically 2–5 lobes connected by thin chromatin strands)
- Dark purple** and densely stained
- The nucleus of a mature neutrophil often resembles a **croissant or twisted ribbon**, which makes it easy to recognize once you're familiar with the shape.

LABORATORY 10 (IN PERSON): RNA EXTRACTION FOR QRT-PCR

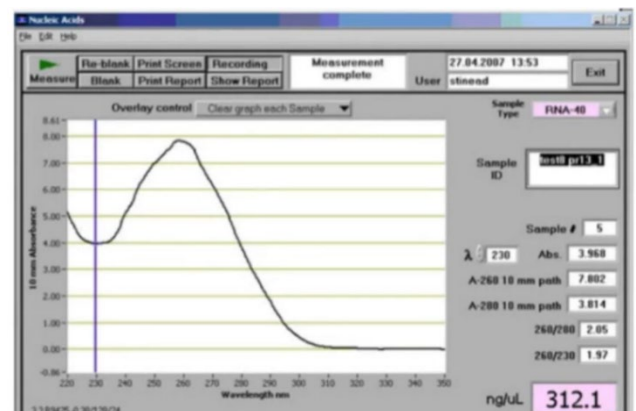
POLYMERASE CHAIN REACTION (PCR)

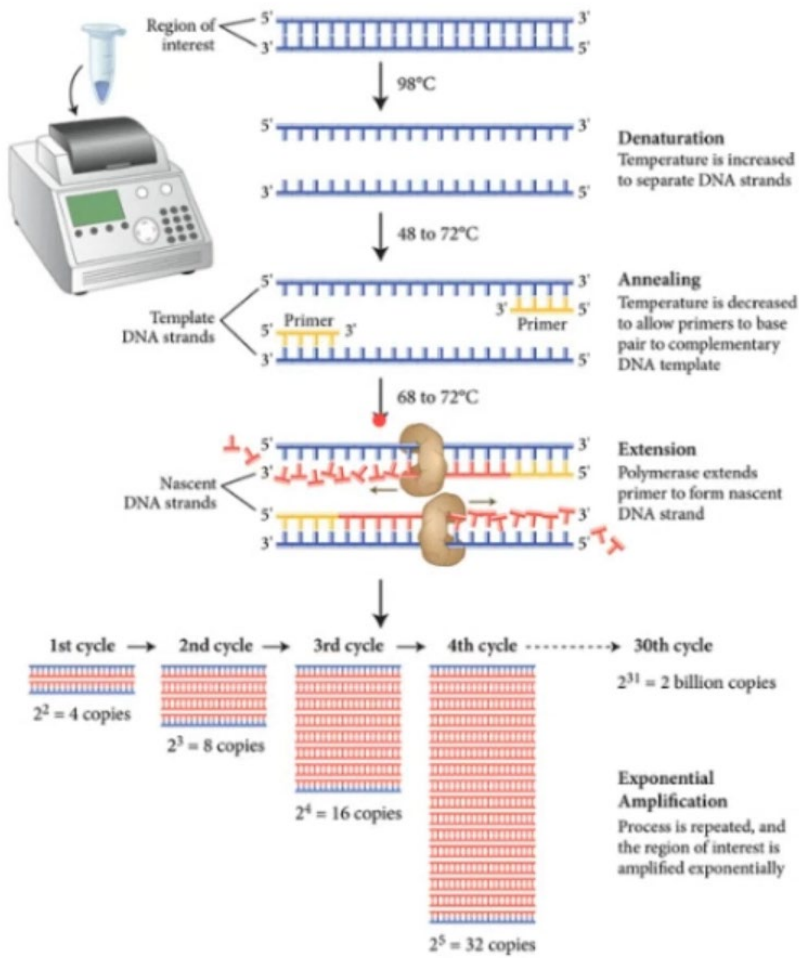
DNA/RNA Extraction

- Tissue flash frozen in liquid nitrogen or immersed in extraction solution.
- RNA extraction yield can be enhanced by
 - Decontaminating working areas with RNaseZap
 - Freezing tissue rapidly – every second not frozen is more RNA that is lost!
 - Use RNA preservation buffer like RNAlater
- Tissue homogenized and DNA/RNA extracted using commercially available kits
- Nucleic acid quality and quantity measured by a spectrophotometer
 - Nucleic acids absorb light at 260nm, contaminants such as phenol and protein (280 nm) are assessed
 - Since RNA and DNA absorb at 260, RNA extractions require DNase, and DNA extractions require RNase.
- Separation by gel electrophoresis is also commonly used to assess DNA size, quantity, and quality.

Polymerase Chain Reaction (PCR)

- DNA extracted from sample and stored at -20C
- PCR requires DNA, primers flanking the gene of interest (conferring sequence specificity), heat resistant polymerase (Taq), and nucleotides (dNTPs)
- Taq and dNTPs in buffer (called master mix) are incubated with forward and reverse primers and sample DNA and placed into thermocycler
 - Strands melted at ~95C
 - When temperature decreases, primers anneal with complementary DNA





- Taq polymerase extends primer and replicates target sequence
- 30 cycles later you have 1 billion copies
- Sources of error include
 - Mispriming – Primers bind to off target sequence and prime replication
 - Secondary DNA structure can obstruct primer recognition
 - Primers dimerize and amplified (primer dimer)

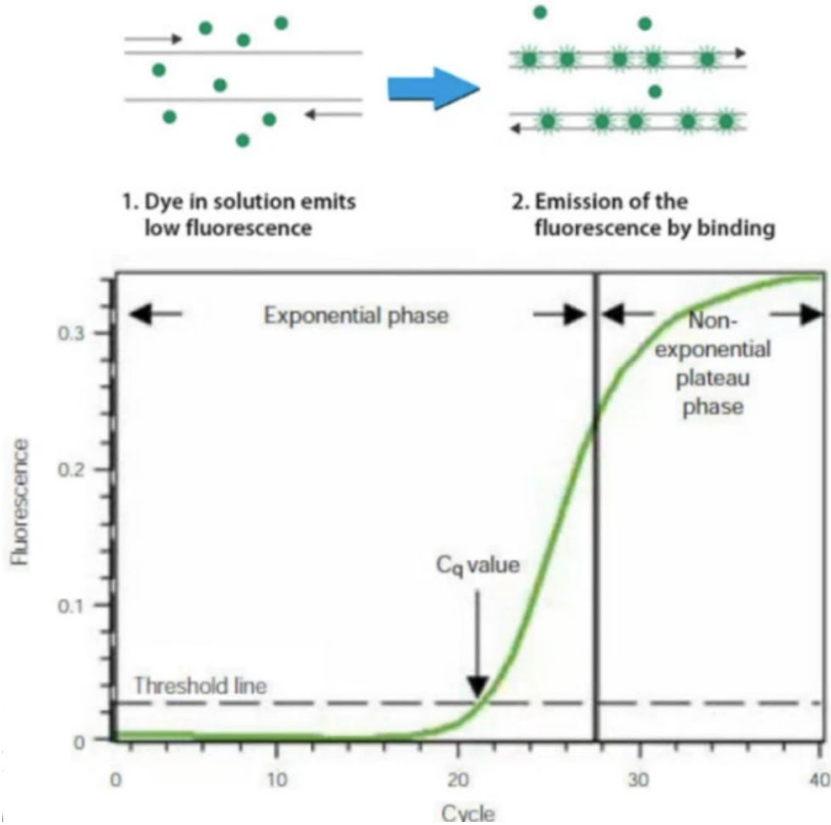
QUANTITATIVE REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (QRT-PCR)

Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR)

- RNA from sample is reverse transcribed into cDNA for PCR reaction:
 - RT also requires primers. Either a gene specific primer is used or random primers + PolyA.
 - Amount of starting material is critical!
- PCR reaction coupled with fluorescent molecule that emits light when bound to double stranded DNA.
 - SYBR green is commonly used. Safer and brighter than ethidium bromide.
 - SYBR green fluorescence increases 100-fold when bound to double strand DNA.
- As the amount of dsDNA increases exponentially during PCR, so does fluorescence
 - Fluorescence is measured by special thermocycler that can excite and quantify probe fluorescence
- The rate of exponential growth depends on the amount of starting materials (more RNA = less cycles for detection)

RT-qPCR Workflow

1. Order 18-22 bp primers specific for your gene of interest
 - a) BLAST can be used to check for off-target homology
 - b) Amplicons should be 100-200 bp
2. Extract total RNA (or mRNA), treat with DNase, and reverse transcribe into cDNA
 - a) Gene specific primer, reverse transcriptase, RNA, dNTPs
 - b) Random primers plus PolyT tail primer can be used to amplify all mRNA if more than one gene will be interrogated in the sample.
3. Residual RNA degraded with RNase
 - a) cDNA can be stored for months at -20C
4. Make master mix and place test tube on ice.
 - a) cDNA from steps 1-3, Taq polymerase, dNTP mix, forward and reverse primers, SYBR green
5. Place master mix into real-time thermal cycler and monitor reaction.



RT-QPCR DATA ANALYSIS

- Schmittgen TD and Livak KJ. Analyzing real-time PCR data by the comparative C_T method. Nature Protocols 2008 Jun 5;3:1101-1108.**
[https://www.nature.com/articles/nprot.2008.73#:~:text=Real%2Dtime%20PCR%20data%20has,%CE%94%CE%94%20C%20T%20method\)15](https://www.nature.com/articles/nprot.2008.73#:~:text=Real%2Dtime%20PCR%20data%20has,%CE%94%CE%94%20C%20T%20method)15)

RT-PCR data analysis involves quantifying nucleic acids, typically mRNA, to assess gene expression or detect pathogens. Two main methods are employed: absolute quantification and relative quantification. Absolute quantification determines the precise number of target copies, often by using a standard curve. Relative quantification compares the expression of a target gene to a reference gene, or between different samples. Various analytical methods, including the comparative C_T method ($2^{-\Delta\Delta CT}$), are used to interpret the data.

Here's a more detailed breakdown:

1. Data Acquisition and Visualization:

Real-time PCR generates amplification plots, showing the increase in fluorescence signal as PCR progresses. These plots are typically S-shaped curves, with a baseline, a log-linear phase, and a plateau phase. Data analysis software provides visualizations like amplification plots, melt curves, and scatter plots.

2. Key Parameters in RT-PCR Analysis:

CT (Cycle Threshold):

The PCR cycle number at which the fluorescence signal crosses a set threshold.

PCR Efficiency:

The rate at which the target DNA is amplified in each cycle.

Threshold:

A critical parameter in RT-PCR data analysis. It should be set high enough to avoid background noise but low enough to capture the log-linear phase of amplification.

Baseline:

The initial phase of the amplification curve where fluorescence signal is minimal.

3. Absolute Quantification:

Involves creating a standard curve using known concentrations of a target template.

The CT values of unknown samples are then compared to the standard curve to determine the initial copy number of the target.

4. Relative Quantification:

ΔC_{T} (Delta C_{T}):

Calculated by subtracting the CT of a reference gene from the CT of the target gene in the same sample.

$\Delta\Delta C_{T}$ (Delta Delta C_{T}):

Calculated by subtracting the ΔC_{T} of a control sample from the ΔC_{T} of a treatment sample.

$2^{-\Delta\Delta C_{T}}$:

A common formula used to calculate the relative change in gene expression between two samples.

5. Data Analysis Methods:

Efficiency-Calibrated Model: Takes into account the amplification efficiency of each primer set.

$\Delta\Delta C_{T}$ Method: A simplified method that assumes similar amplification efficiencies between target and reference genes.

Statistical Analysis: Includes t-tests, ANOVA, and other methods to assess statistical significance of differences in gene expression.

6. Software and Tools:

Real-time PCR instruments often come with software for data analysis.

Specialized software packages like DataAssist Software and the GeneGlobe Data Analysis Center offer tools for various analysis tasks.

Software packages like rtpcr in R also provide statistical analysis capabilities.

7. Quality Control:

It's crucial to ensure data quality by setting appropriate thresholds, correcting for background fluorescence, and assessing amplification efficiencies.

Replicates and controls are essential for reliable data.

Statistical analysis should be carefully considered, especially with low-quality data.

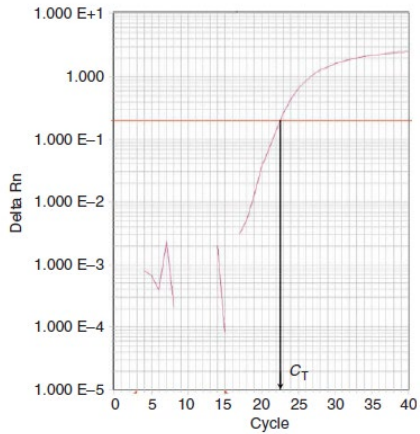


Figure 1 | Real-time PCR output, calculation of C_T . Data is presented from a typical real-time PCR output. The PCR was run for 40 cycles. The point at which the curve intersects the threshold (horizontal red line) is the C_T . The C_T in this example is 22.5.

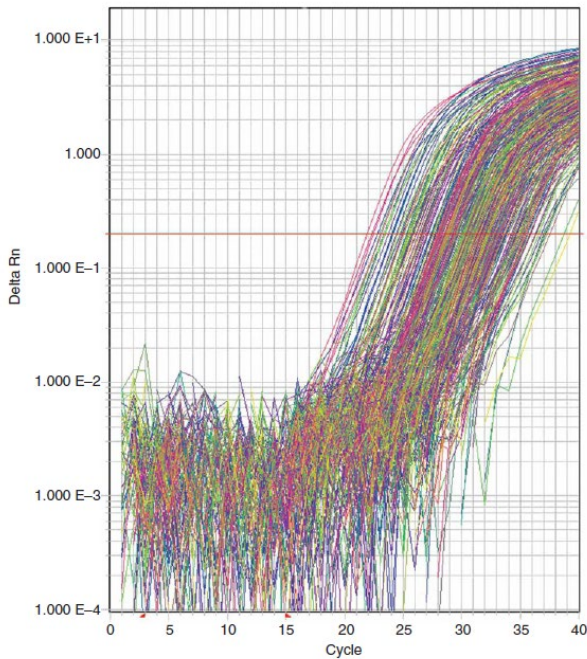


Figure 2 | Real-time PCR plots from gene expression profiling. The expression of 366 different genes was profiled in the identical sample of cDNA. The shapes of the amplification plots are similar, demonstrating similar PCR efficiency.

LABORATORY 11 (IN PERSON): IN LAB PROPOSAL PREPARATION AND CULTURE OF 32DCL3 CELLS

See laboratory 7 for instructions on culturing 32Dcl3 cells.

In laboratory research proposal preparation in groups.

LABORATORY 12 (IN PERSON): FLOW CYTOMETRY I / CD11B STAINING OF 32DCL3 CELLS UNDERGOING MYELOID DIFFERENTIATION

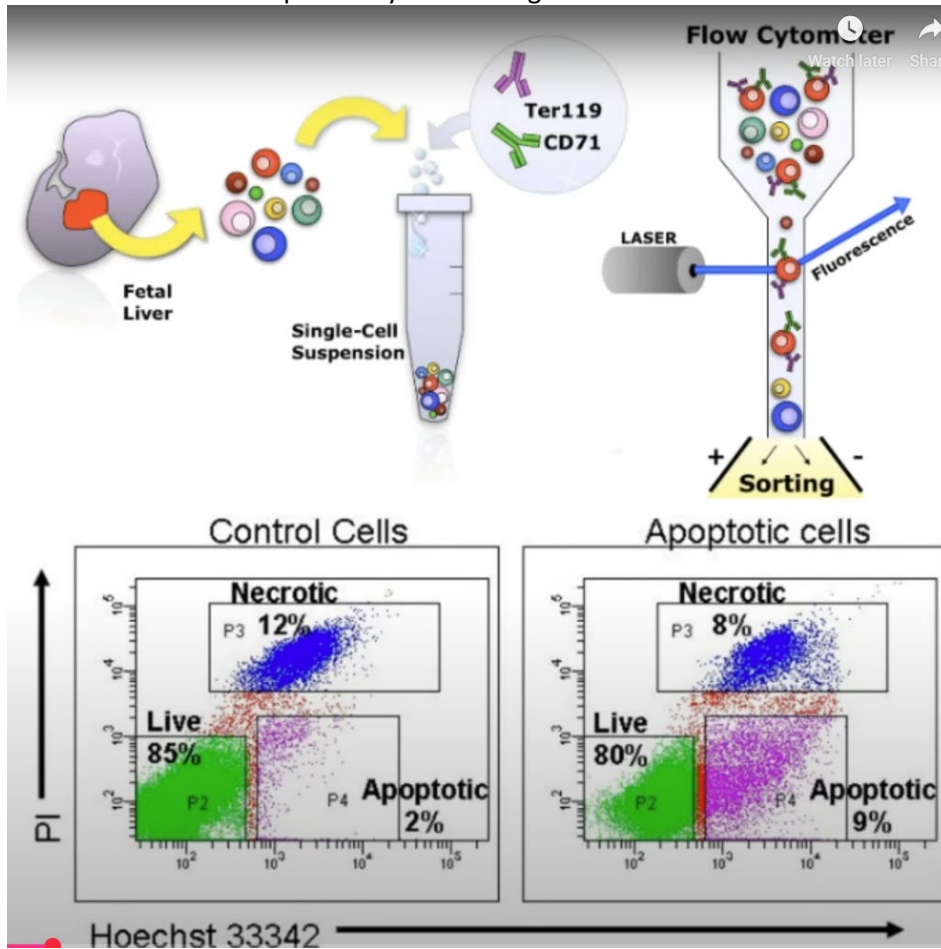
FLOW CYTOMETRY INTRODUCTION - BASICS

Cytometry: The measurement of cell characteristics, including cell size, cell count, cell cycle, and more.

Flow Cytometry: A cell analysis technique to measure the volume of cells in a rapidly flowing fluid stream as they pass in front of a viewing aperture.

Flow cytometry is a laser-based technique used to analyze the physical and chemical characteristics of cells and other particles in a fluid. It works by passing individual cells through a laser beam and measuring how the light scatters and how much fluorescence is emitted by each cell. This information is used to identify, count, and characterize cells, as well as to analyze their properties.

- Cell suspension placed into flow cytometer that funnels cells into a thin tube by a process called hydrodynamic focusing
 - Cells form a single file line for subsequent analysis by the laser.
- Droplets containing single cells are dropped past a detection laser
 - Forward Scatter Light – Measures cell size
 - Side Scatter Light – Measures cell complexity
- Cell suspension input can either be genetically engineered to contain fluorescent protein, or pre-incubated with antibodies towards surface receptors.
- Fluorescent cells are captured by electromagnetism.

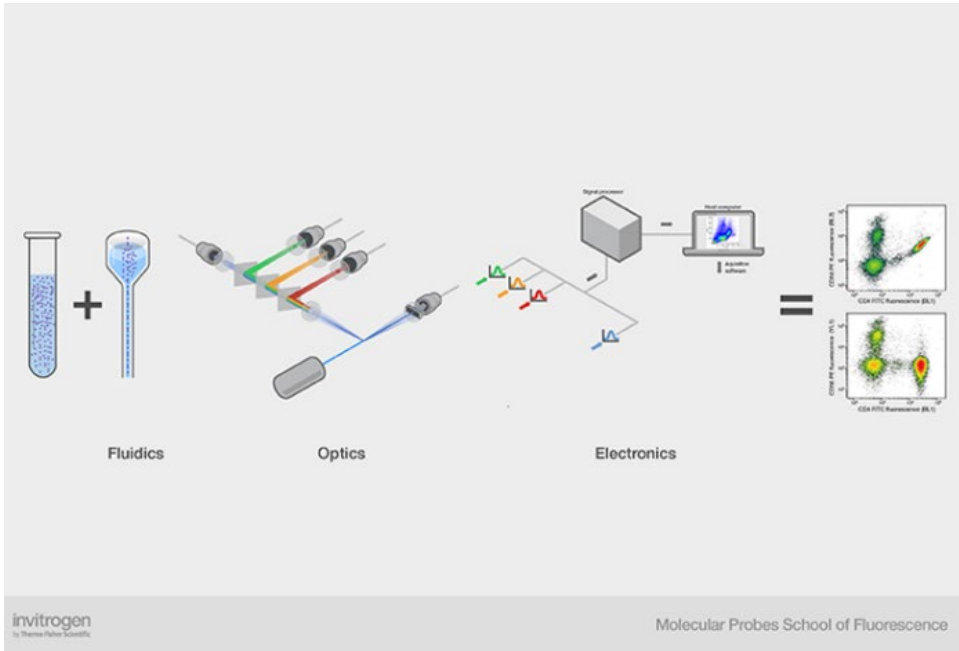


Potential Applications for Flow Cytometry:

- Protein expression
- Protein post-translational modifications
- RNA expression (e.g. mRNA, microRNA, lncRNA)
- Cell health status (e.g. viability, apoptosis, programmed cell death)

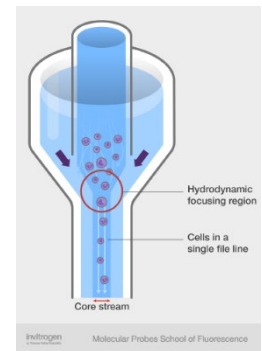
- Cell cycle status (e.g. G0/G1, G2, S, polyploidy)
- Identification and characterization of distinct subsets of cells within a heterogeneous sample
- Cell sorting

Overview of How a Flow Cytometer Works:



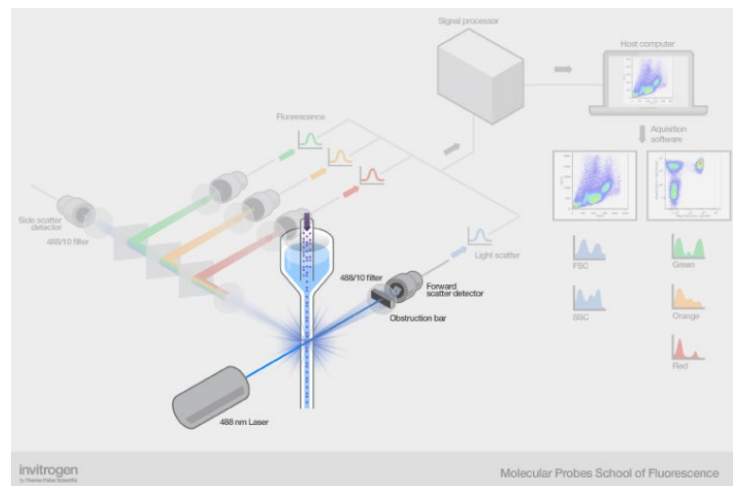
Sample uptake into the instrument:

1. Samples containing cell suspensions or particles are taken up into the instrument.
2. The cells/particles are surrounded by a physiological buffer called sheath fluid.
3. The fluidics system (including tubing, pumps, and valves) organizes the initial sample suspension into a single-file stream of cells.

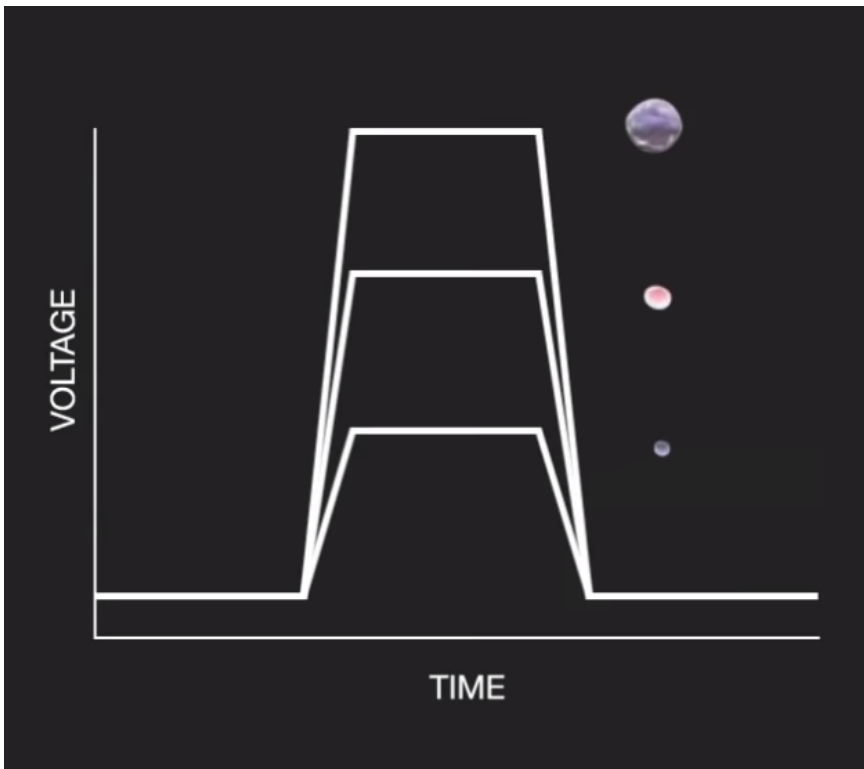


Interrogation Point (Laser Intercept):

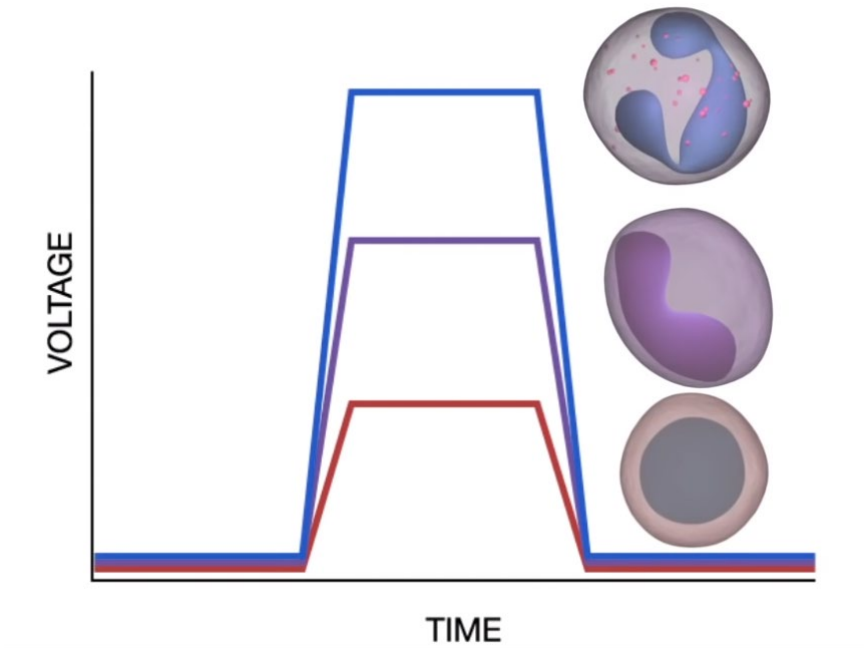
1. The interrogation point, aka laser intercept, is the place where cells interact with laser light.
2. Laser light illuminates a single cell, and some of the light scatters:
3. Forward Scatter (FSC)
4. Side Scatter (SSC)
5. Fluorescence intensity will also be detected.



Forward scatter, or FSC, indicates the size of the cell. As you can see here, smaller cells have less forward scatter than intermediate and large sized cells.



Side scatter, or SSC, indicates the shape and internal complexity of the cell. Lymphocytes, which have a large nucleus and not much granularity give less side scatter than more complex cells, such as macrophages or neutrophils, that have a multilobed nucleus and lots of intracellular granules.



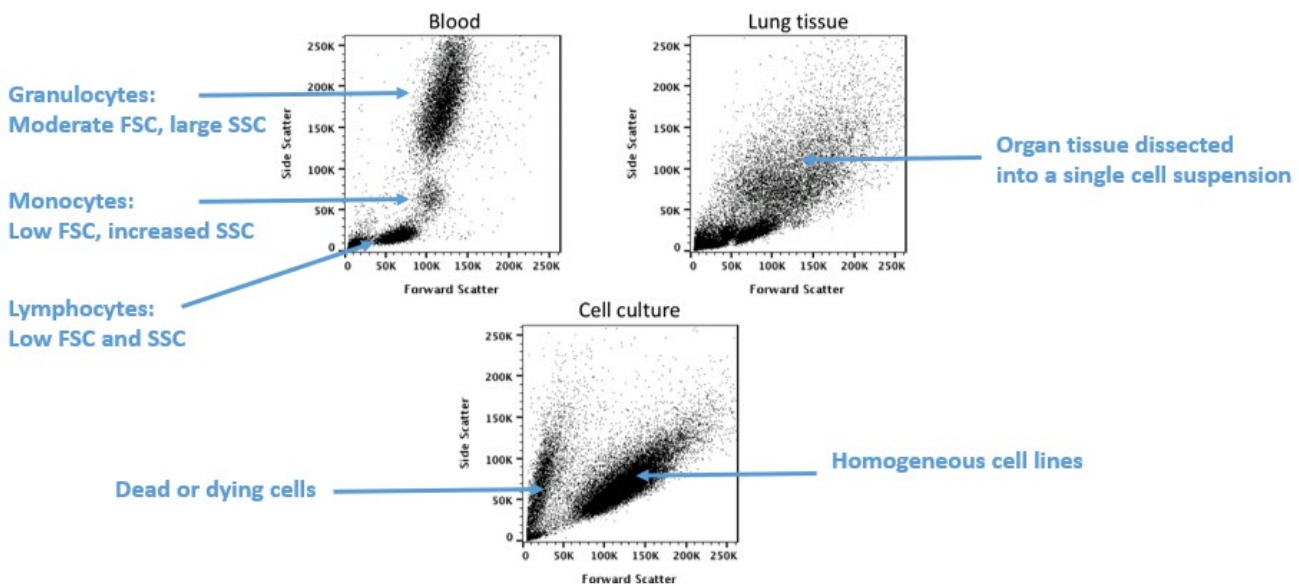
If we look at scatter plots for different types of cell preparations, you'll notice they look very different.

If we look at **blood**, without any fluorescent staining we can already detect different populations of cells. For instance, the lymphocytes are relatively small and have a low forward and side scatter. Monocytes, on the other hand, are still relatively small, but demonstrate increased side scatter. Then there are granulocytes that are a bit larger and have much higher side scatter due to their contents and shape.

On the other hand, if we look at **organ tissue** that has been dissected into a single cell suspension, you can see we still have lymphocytes here, with low forward and side scatter, but the rest of what we can see looks very ambiguous and complex, and is likely many, many different types of cells.

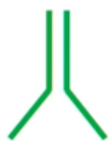
There are also **cell lines**, which are very homogenous, and will generally show up in roughly the same location every time. The material on the left is dead or dying cells or debris.

Differentiating Cell Types



Fluorescence

- Classic flow cytometry uses very specific antibodies to label cellular antigens (extracellular or intracellular).
- The antibodies are directly conjugated in most cases with fluorescent dye(s) to generate a fluorescent signal.



Antibody1-FITC



Antibody2-PE



Antibody3-PECy7

CD11B STAINING AND FLOW CYTOMETRY

Description

CD11b is a 170 kD glycoprotein also known as α M integrin, Mac-1 α subunit, Mol, CR3, and Ly-40. CD11b is a member of the integrin family, primarily expressed on granulocytes, monocytes/macrophages, dendritic cells, NK cells, and subsets of T and B cells. CD11b non-covalently associates with CD18 (β 2 integrin) to form Mac-1. Mac-1 plays an important role in cell-cell interaction by binding its ligands ICAM-1 (CD54), ICAM-2 (CD102), ICAM-4 (CD242), iC3b, and fibrinogen.

Cell Surface Flow Cytometry Staining Protocol

Reagent List

- Cell Staining Buffer (BioLegend Cat. No. [420201](#))
- Red Cell Lysis Buffer (BioLegend Cat. No. [420301](#))
- 7-AAD Viability Staining Solution (BioLegend Cat. No. [420403](#))
- TruStain FcX™ (anti-CD16/32, BioLegend Cat. No. [101319](#))
- Human TruStain FcX™ (Fc Receptor Blocking Solution, BioLegend Cat. No. [422301](#))
- TruStain FcX™ PLUS (anti-CD16/32, recommended for mouse cells. BioLegend Cat. No. [156603](#))

Protocol Steps

Harvest Tissue or Cells:

1. Obtain desired tissue (e.g. spleen, lymph node, thymus, bone marrow) and prepare a single cell suspension in Cell Staining Buffer (BioLegend Cat. No. 420201). If using in vitro stimulated cells, simply resuspend previously activated cultures in Cell Staining Buffer and proceed to Step 2.
2. Add Cell Staining Buffer up to ~15ml and centrifuge at 350xg for 5 minutes, discard supernatant.

Lyse Red Cells:

3. If necessary (e.g. spleen), dilute 10X Red Blood Cell (RBC) Lysis Buffer (BioLegend Cat. No. 420301) to 1X working concentration with DI water and resuspend pellet in 3 ml 1X RBC Lysis Buffer. Incubate on ice for 5 minutes.
4. Stop cell lysis by adding 10ml Cell Staining Buffer to the tube. Centrifuge for 5 minutes at 350xg and discard supernatant.
5. Repeat wash as in step 2.
6. Count viable cells and resuspend in Cell Staining Buffer at 5-10 x 10⁶ cells/ml and distribute 100 μ l/tube of cell suspension (5-10 x 10⁵ cells/tube) into 12 x 75mm plastic tubes.

Block Fc-Receptors:

Reagents that block Fc Receptors may be useful for reducing non-specific immunofluorescent staining.

Note: Mouse TruStain FcX™ PLUS contains antibodies directed against CD16/32 (via the Fab portion of the antibody), while Human TruStain contains specialized human IgG that bind to Fc receptors via the Fc portion of the antibodies. Human TruStain is compatible with flow cytometric staining with anti-human CD16 (clone 3G8), CD32 (clone FUN-2), and

CD64 (clone 10.1) antibodies.

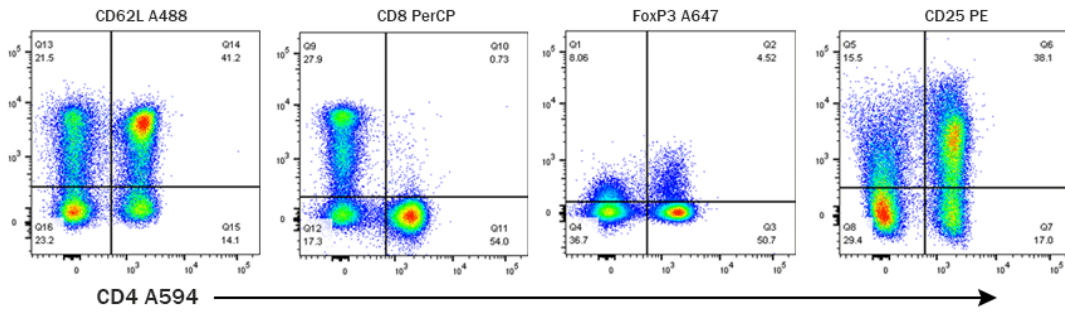
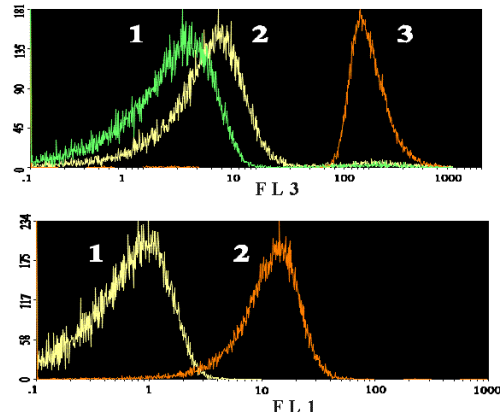
7. For mouse samples, we recommend using TruStain FcX™ PLUS (anti-mouse CD16/32) Antibody specific for FcγR III/II (Cat. No. 156603, clone S17011E). Block Fc receptors by pre-incubating cells with 0.25µg of TruStain FcX™ PLUS (anti-mouse CD16/32) Antibody per 10⁶ cells in a 100µl volume for 5-10 minutes on ice.
Note: For step 7, 1µg of TruStain FcX™ (anti-mouse CD16/32) Antibody (Cat. No. 101319, clone 93) per 10⁶ cells in a 100µl volume can be used. However, we strongly recommend using TruStain FcX™ PLUS as indicated, based on in-house testing results that demonstrate its superior blocking capabilities.
8. In humans, cells can be pre-incubated with 5µl of Human TruStain FcX™ (Fc Receptor Blocking Solution, BioLegend Cat. No. 422301) per 100µl of cell suspension for 5-10 minutes at room temperature. In the absence of an effective/available blocking antibody, an alternative approach is to pre-block cells with excess irrelevant purified Ig from the same species and same isotype as the antibodies used for immunofluorescent staining.

Cell-Surface Staining with Antibody:

9. Add appropriately conjugated fluorescent, biotinylated, or purified primary antibodies at predetermined optimum concentrations (e.g. anti-CD3-FITC, anti-CD4-Biotin, and anti-CD8-APC) and incubate on ice for 15-20 minutes in the dark.
10. Wash 2X with at least 2ml of Cell Staining Buffer by centrifugation at 350xg for 5 minutes.
11. If using a purified primary antibody, resuspend pellet in residual buffer and add previously determined optimum concentrations of anti-species immunoglobulin fluorochrome conjugated secondary antibody (e.g. FITC anti-mouse Ig) and incubate on ice in the dark for 15-20 minutes.
12. Repeat step 10.
13. Resuspend cell pellet in 0.5ml of Cell Staining Buffer and add 5µl (0.25µg)/million cells of 7-AAD Viability Staining Solution (BioLegend Cat. No. [420403](#)) to exclude dead cells.
Note: BioLegend recommends using the Spectra Analyzer to decide compatibility with other fluors.
14. Incubate on ice for 3-5 minutes in the dark.
15. Perform fluorescence activated cell sorting (FACS), or flow cytometric analysis.
Note: If you are unable to immediately read your samples on a cytometer, keep them shielded from light and in a refrigerator set at 4-8°C. The samples should be resuspended in Cell Staining Buffer. Note that samples should not remain in a fixation buffer for extended periods of time as this can affect fluor conformation and fluorescence.

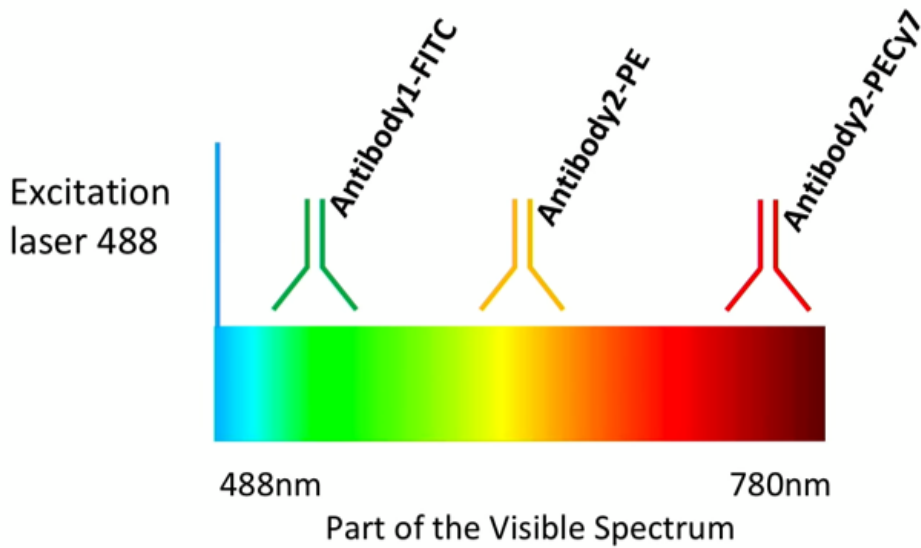
LABORATORY 13 (IN PERSON): FLOW CYTOMETRY II / ANNEXIN V STAINING OF 32DCL3 CELLS UPON IL-3 WITHDRAWAL

FLOW CYTOMETRY INTRODUCTION - ADVANCED



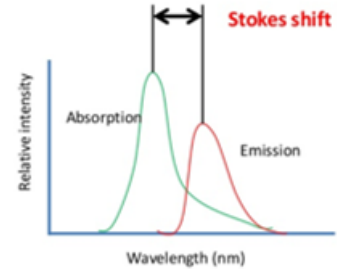
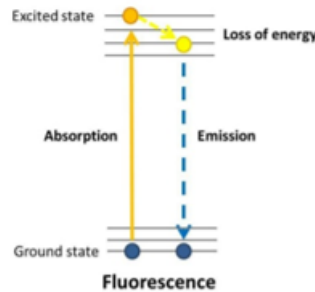
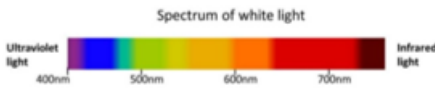
Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation. Fluorescent dyes or fluorescent labeled antibodies are used to stain a suspension of cells.

Antibodies Conjugated to Fluorescent Dyes



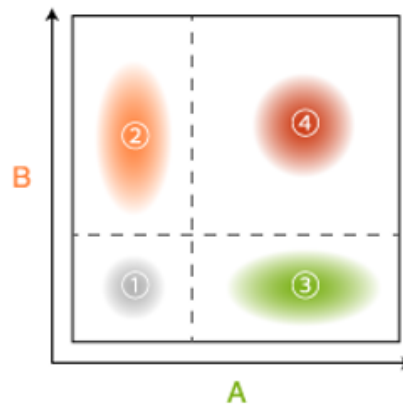
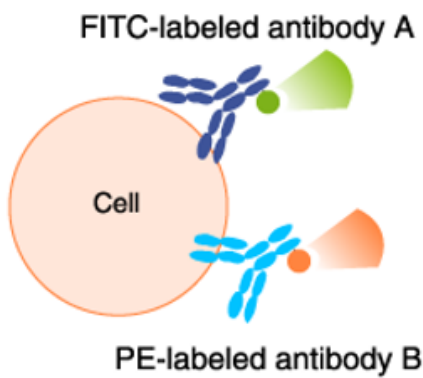
Fluorescence

- Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation.



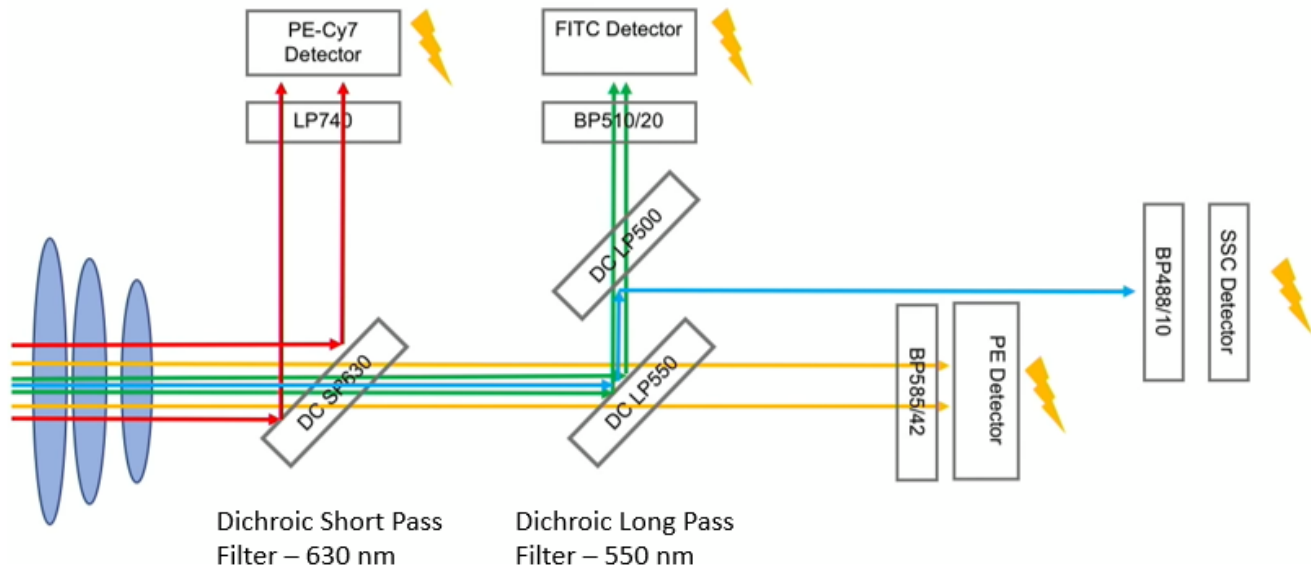
- Fluorescent dyes or fluorescently labeled antibodies are used to stain a suspension of cells

Detecting Fluorescence



- 1) Cells not expressing either A or B
- 2) Cells expressing only B
- 3) Cells expressing only A
- 4) Cells expressing both A and B

Detecting Fluorescence



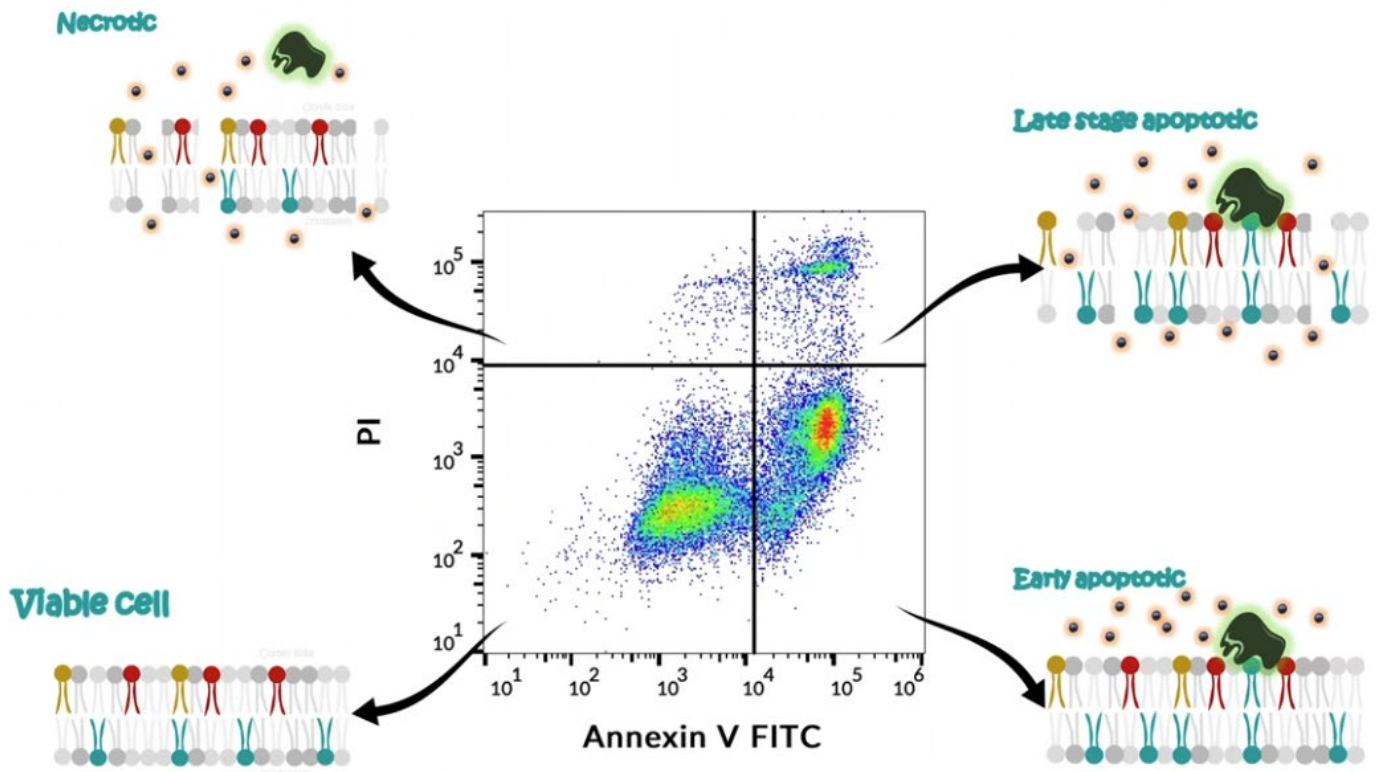
ANNEXIN V STAINING AND FLOW CYTOMETRY

Description

Annexin V (or Annexin A5) is a member of the annexin family of intracellular proteins that binds to phosphatidylserine (PS) in a calcium-dependent manner. PS is normally only found on the intracellular leaflet of the plasma membrane in healthy cells, but during early apoptosis, membrane asymmetry is lost and PS translocates to the external leaflet. Fluorochrome-labeled Annexin V can then be used to specifically target and identify apoptotic cells. Annexin V Binding Buffer (cat. no. 422201) is recommended for use with Annexin V staining. Annexin V binding alone cannot differentiate between apoptotic cells and necrotic. Therefore, we recommend using our Helix NP™ Blue (Cat. No. 425305), Helix NP™ Green (Cat. No. 425303) or Helix NP™ NIR (Cat. No. 425301). Early apoptotic cells will exclude 7-AAD and PI, while late stage apoptotic cells and necrotic cells will stain positively, due to the passage of these dyes into the nucleus where they bind to DNA.

Annexin V Staining

1. Wash cells twice with cold BioLegend Cell Staining Buffer (Cat. No. 420201) and then resuspend cells in Annexin V Binding Buffer (Cat. No. 422201) at a concentration of 1×10^6 cells/mL.
2. Transfer 100 μ L of cell suspension in 5 mL test tube.
3. Add 5 μ L of fluorochrome conjugated Annexin V.
4. Stain with a viability dye, such as PI (Cat. No. 421301), 7-AAD (Cat. Nos. 420403 & 420404), or Helix NP dyes (Cat. Nos. 425301, 425303, & 425305), if desired.
5. Gently vortex the cells and incubate for 15 min at RT (25°C) in the dark.
6. Add 400* μ L of Annexin V Binding Buffer (Cat. No. 422201) to each tube. *For more concentrated samples, add a minimum of 200 μ L of Annexin V Binding Buffer in this step.
7. Analyze by flow cytometry.



LABORATORY 14 (IN PERSON): THANKSGIVING – NO CLASS!!!

Happy Thanksgiving!! 😊

LABORATORY 15 (IN PERSON): RESEARCH PROPOSAL CHALK TALKS

Get ready to convince other students that your proposal is worth doing!!!!

LABORATORY 16 (IN PERSON): FINAL PRESENTATIONS

Final proposals due and group presentations.

APPENDIX I: GENERAL PROCEDURES FOR CONDUCTING EXPERIMENTS

Preparing for a laboratory experiment requires careful planning and adherence to safety protocols. Here are some good procedures to follow in a teaching laboratory:

1. PRE-CHECK

- **Review Experiment Details:** Thoroughly read and understand the experiment's protocol, objectives, and any specific instructions provided by the protocol provided, your instructor or lab manual. Make sure you have a clear idea of what you will be doing, what materials, you'll need, what roles need to be filled if you work in a team, what is required of each role.
- **Communication:** If you are working with others, communicate clearly about each person's role and responsibilities. Ensure everyone understands the experiment's objectives and procedures.
- **Data Analysis:** If data analysis is part of the experiment, prepare any necessary spreadsheets or analysis tools in advance.
- **Record Keeping:** Have a dedicated lab notebook to record your observations, methods, and results. Make sure your notebook is well-organized and compliant with your lab's record-keeping guidelines. This is a good time to have your notebook ready or any material ready for data collection and write-up for observations.

2. RAMP UP: RECOGNIZE HAZARDS, ASSESS THE RISKS OF HAZARDS, MINIMIZE THE RISKS OF HAZARDS, PREPARE FOR EMERGENCIES

- **Wear Appropriate Attire:** Dress in laboratory-appropriate attire, including a lab coat, closed-toe shoes, and safety goggles. Long hair should be tied back, and loose clothing should be avoided.
 - **Personal Protective Equipment (PPE):** Put on any required PPE, such as gloves or a face mask, as specified in the lab protocol.
- **Safety Check:** Familiarize yourself with the location of safety equipment in the lab, including eyewash stations, fire extinguishers, and emergency exits. Know how to use them in case of an emergency.
- **Review Safety Procedures:** Review and follow all safety protocols relevant to your experiment. Understand the potential hazards and how to mitigate them. Review the Safety Data Sheets (SDS) for any hazardous chemicals you'll be using.

3. PLAN YOUR WORKFLOW:

- **Review Protocols Again:** Before starting the experiment, briefly review the protocol to ensure you haven't missed any critical steps. Plan the sequence of steps you will follow during the experiment, including any incubation or waiting periods. This helps you work efficiently and minimizes errors. You may include workflow-charts or a diagram to help guide your work-flow.

- **Sanitize Your Work Area:** Clean your workspace with 70% ethanol or an appropriate disinfectant to minimize contamination risks. Ensure your workbench is uncluttered and ready for your experiment.
- **Gather, Equipment, Materials and Reagents:** Before starting, ensure you have all the necessary materials, including glassware, pipettes, agar plates, media, chemicals, and any specialized equipment. Check that your supplies are in good condition and not expired.
 - **Prepare Solutions:** If your experiment requires the preparation of solutions or media, make sure you have them ready in the correct concentrations and volumes.
 - **Calibrate Equipment:** If you'll be using any lab equipment, such as microscopes, balances, or pipettes, ensure they are calibrated and functioning correctly.
 - **Label Everything:** Label your tubes, plates, and containers with your name, date, and any other necessary identifiers. This helps prevent mix-ups and ensures accurate data recording.

4. DURING THE EXPERIMENT

- **Maintain Sterility:** If your experiment requires sterile conditions, take extra precautions, such as working within a laminar flow hood or using aseptic techniques.

5. WRAP-UP: FINISH NOTES, CLEAN-UP, DECONTAMINATE!

- **Dispose of Waste:** Understand the proper disposal procedures for waste materials, including contaminated media, cultures, or chemicals. Use designated waste containers.
- **Clean Up:** After completing the experiment, clean and disinfect your workspace, including any equipment you used. Return materials and equipment to their designated storage locations.
- **Data Analysis:** If data analysis is part of the experiment, prepare any necessary spreadsheets or analysis tools in advance.
- **Ask for Help:** If you are uncertain about any aspect of the experiment or safety procedures, don't hesitate to ask your instructor or a lab supervisor for guidance.

6. POST-EXPERIMENT FOLLOW-UP