Fall 9-1-2021

BIOB 411.01: Immunology Laboratory

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BIOB 411 IMMUNOLOGY LABORATORY

Autumn Semester, 2021

Dr. Scott Wetzel (CHCB 216; Phone 243-2168, scott.wetzel@umontana.edu)
Lab schedule: 3:00 - 5:50 pm on Thursday in HS 404 and 405
Teaching Assistant: Polash Biswas

Date	Lab #	Page	Topic
September			
2	**1**	**1**	Introduction - grading, animal use, safety, etc.
9	**2**	**10**	Cells and organs of the immune system- PART 1
16	**3**	**18**	Cells and organs of the immune system- PART 2
23	**4**	**25**	Generating polyclonal antibodies- PART 1
30	**5**	**28**	Immunoagglutination assays
October			
7	**6**	**33**	Immunoprecipitation and Electrophoresis
14	**7**	**40**	Complement fixation assay
21	**8**		**MIDTERM EXAM**
28	**9**	**43**	Passive hemagglutination & Generating polyclonal antibodies – PART 2
November			
4	**10**	**48**	Flow Cytometry ---------- **LAB PAPER OUTLINE DUE**
11	**11**		**No Class – Veteran's Day Holiday**
18	**12**	**51**	ELISA
25	**13**		**No Class – Thanksgiving Holiday**
December			
2	**14**	58	Generating polyclonal antibodies- PART 3
9	**15**	61	Complete analysis of antisera, compile data and Lab Paper
15	-		**FINAL EXAM (3:20 – 5:20)**
17	-		**LAB PAPER & NOTEBOOKS DUE by 5:00 PM**

General Notes:

GRADING- BIOB 411 points are broken down as follows:

200 pts. from 2 lab exams (midterm and final)

100 pts. from a formal lab report (Instructions below – Due December 17)

60 pts. from the lab notebook (5 pts. per lab)

30 pts. from quizzes

10 pts. from detailed lab report outline (due November 4)

Grades are calculated by 90%, 80%, 70%, and 60% of the total points; which represent the cutoffs for an A, B, C, and D, respectively.

If Lab Manuals or the Final Lab Report are turned in late for grading, they will be penalized by losing 1 letter grade (10% of points possible) per day of tardiness.

There are no makeup labs available and Students missing more than two labs will be required to drop the course.

QUIZZES: These are long, and frequently complex laboratory exercises. You **MUST** read the material and prepare for each lab **AHEAD** of time (remember this is a 2-credit course). To ensure that you come prepared, we will have unannounced quizzes at the beginning of 6 of the first 12 labs during the semester. These quizzes will be worth 5 points and will cover the lab that day. Quizzes will be administered via Moodle.

LAB PAPER- Each team will receive a mouse for generating "monospecific" polyclonal antiserum. The protocol and results obtained from this portion of the laboratory (labs 4, 9, 12 & 13) will be summarized by each student in a typewritten report. The report format will follow the publication style as set forth by the *Journal of Immunology* (for details see Laboratories 13 & 14). **Late papers WILL NOT BE ACCEPTED – Papers are due December 17.**

LAB NOTEBOOK- Each student will maintain a personal and comprehensive lab notebook. Loose-leaf paper is **NOT** acceptable. Record your data and observations **IN PEN** for future reference. Be sure and take your notes **DURING** the lab. Evidence of post-lab entry of observations will lower your grade. Please note that lab notebooks will be picked up periodically during the semester for grading by the TA. **Late notebooks WILL LOSE 1 LETTER GRADE PER DAY and WILL NOT BE ACCEPTED AFTER December 17.**

General Info: Keeping a Laboratory Notebook

All students will be **required** to maintain a laboratory notebook. The notebook will be used for the recording of laboratory data and calculations, and will be critically important for writing your lab paper. It will also be periodically picked up and graded.

The purpose of a laboratory notebook is to allow anyone with some knowledge to understand **exactly** what you did. You need to record the information in sufficient detail so as to be able to repeat it, and you must be able to understand exactly what your results were. You will need good notes to be able to write your lab reports; in addition, your notebook may allow you to figure out why some parts of your experiments did not work as expected.

Companies that perform research require their employees to keep proper notebooks. In these companies, company policy dictates that any work not recorded in the notebook was never actually performed. As a result, the work must be repeated, which tends to have deleterious effects on the career opportunities of the employees involved. In cases of disputes as to priority, notebook dates are sometimes used to indicate exactly when an experiment was performed. Ownership of patents (and in some cases large amounts of money) can therefore be critically dependent on keeping a proper notebook. Instruction in keeping laboratory notebooks is therefore a major part of most laboratory courses.

In your notebook, each experiment should begin with a **title**, a **date**, and a statement of the **objective(s)** of the planned work; in other words, an introduction. You should also **record exactly what you did at each step** (being sure to mention anything that you did that differed from the information in the Manual). In addition, you should record any numerical information,

such as the weights of reagents used, absorbance readings, protein concentrations, and buffer concentrations.

Everything you do should be recorded **directly** into your lab notebook in **pen**. If you make a mistake, draw a line through it, and write the correction next to the mistake. (It may turn out that the original information was correct after all, so do not obliterate the original information by erasing it, or by removing the page from your notebook.) Any calculations performed should be written directly into your book. Hard copies of work done on a computer and printouts from laboratory instruments should be taped directly into your lab notebook.

Writing important information on scrap paper, and then recording it in your notebook later is **not** acceptable. **If you are writing something while in the laboratory, you should be writing it directly into your notebook.**

At each step in your experiment, in addition to the results, record your thoughts regarding the experiment and how you think it is going. Record your mistakes, and your attempts to rectify them. Record the calculations involved in any type of data analysis, as well as explanations for both what you did and what you think it means. A research project is a journey into the unknown; your laboratory notebook is usually your only guide through the forests of uncertainty.

It is also a good idea to look over your notebook periodically during the semester, and make notes of things that you do not understand, so that you can ask questions before the lab reports are due.

Do not say "well, I will remember what this means"; instead, ***write it down***! Do not say "I will remember what I was thinking while I did this experiment"; instead, ***write it down***! If you use your lab notebook properly, you will find that writing your lab reports is much easier, and you will be developing good habits for the future.

Laboratory Notebook Requirements:

Each experiment in your lab notebook needs to be fully documented and should read like a formal lab paper. These are the required items and their point values for each week's lab period:

Table of Contents
Example:

Table of Contents

In your lab notebook you must have these sections:
Introduction – **(1pt)** – A short paragraph describing the objectives of the lab. Do not just copy what is written in the lab instructions! (you will get a zero if you plagiarize from the lab manual or any other source). You should address: Why did we conduct exercise? What information were we trying to obtain? What concepts were being demonstrated? What protocol or method or technique did we use to obtain the information? The purpose is typically just 1-2 paragraphs, but it should be complete.

Procedure – **(1pt)** Brief outline. You may reference the lab manual, but you **must** include any changes from printed protocol.

Example:
BIOB 411, Immunology Lab, Professor Wetzel, Fall 2021, University of Montana, pages 11-12
Note any deviations from lab manual procedure

Results – **(1pt)** – Numbers, drawings, etc. This is a description of your data and includes any calculations of % difference, etc. that isn't in your figures.

Discussion/Conclusion – **(2 pts)** - What do the results indicate? This is the most important section, explain the reasoning for what the results mean.

Were the objectives listed in the purpose section accomplished? Why or how? What problems were encountered?

Were there any unexpected results? What are possible explanations? Indicate things that might be done differently if the lab were repeated.

Take Home Problems - **(1pt extra credit)** – These will be in your notebook and additional questions will be on the chalkboard.

Final Lab Paper Guidelines:

OUTLINE OF LAB PAPER- Provide an outline of your lab paper to the TA by the due date on page 1 (October 22). Describe your ideas for presenting the data and how you will arrange the results in the context of the format below. This is worth 10 points, so please take the necessary time and effort when preparing this document.

LAB PAPER- The final paper will be typed and double-spaced. The paper is worth ~ 30% of your lab grade. The style of the report follows the format of the *Journal of Immunology* http://www.jimmunol.org/site/misc/authorinstructions.xhtml#generalguide instructions for full-length articles). It is STRONGLY recommended that you read through these directions before writing your paper. The requirements include:

- Title Page- Title, name(s), address(es), key words
- Abstract- A summary of your data (250 words maximum)
- Introduction- the background and justification for your research
- Materials and methods- a reiteration (in your own words) of the labs
- Results- tabulated and graphic data as well as text description. This is a description of your data and includes any calculations of % difference, etc. that isn't in your figures. Make sure you reference your figures.
- Discussion- below

- References- not required for the report, but if you use a reference other than the lab book you MUST provide a reference. (otherwise this will be plagiarism)

GRADING OF THE PAPER IS AS FOLLOWS:

Journal of Immunology format and 5-page limit---- 10 pts. ***(only the first 5 pages will be graded)***

Results---quality and data presentation---30 pts.

Discussion---- 40 pts. Explain what you might have expected and if your results meet those expectations. Tell the significance of the results. Propose future experiments, etc.

Content---20 pts. Grammar, spelling, etc. (this is a 2/3 writing course); reflects the presentation and style.

Please note: You are bound by the University of Montana student conduct code. All work will be performed solely by the student. **Each Student must prepare the final paper individually (this is NOT a joint effort by lab partners)**. If 2 or more identical papers are turned in, we will consider each student to have contributed equally so a single paper will be graded and the final score will be evenly divided between the number of students.

Plagiarism and cheating of any kind will result in referral for disciplinary action and you will receive a zero on the assignment. This will significantly impact your final course grade. All electronic devices (phone, tablet, etc.) are to be POWERED OFF and stored in bags during examination periods. If your phone or other electronic device disrupts the exam in any way, your will lose 25 points from your total grade on that exam – TURN IT OFF!

Students with Disabilities (ODE Accommodations):

The University of Montana assures equal access to instruction through collaboration between students with disabilities, instructors, and Disability Services for Students (ODE http://www.umt.edu/ODE). If you think you may have a disability adversely affecting your academic performance, and you have not already registered with ODE, please contact ODE in Lommasson 154. I will work with you and ODE to provide appropriate accommodation.

Financial Aid Ramifications of Attendance*:*

This is an issue ONLY if you withdraw from the course before the end of the semester. To be eligible to receive federal aid students must participate verifiably at least once in the course, and to be eligible to receive the full amount (whether loans or grants), must have participated through 60% of the course, or roughly the 45th day of classes. We will have an exam on October 21, which will allow me to confirm your participation through the 45th day of classes. Another way is to log into Moodle on or after October 10**.**

Learning Outcomes*:*

- The students should be able to explain the overall functions of the complement cascade, including generation of opsonins, anaphylotoxins, and the membrane attack complex.

- They should be able to differentiate between the classical, alternative and MBL pathways and be able to trace the steps of the various cascades.

- They also need to be able to explain the regulatory mechanisms that control complement activation and diseases, such as hereditary angioedema (HAE), which result of complement dysregulation
- At the end of this series of lectures, the students should be able to distinguish between antigens recognized by B and T lymphocytes, explain conformational versus linear epitopes, and identify characteristics of antigens that increase immunogenicity.
- They should know the structural components of immunoglobulin molecules including the heavy and light chains, F_c, F_{ab}, $F(_{ab}')_2$, C_H domains and the hinge region.
- Student will have a clear understanding and be able to perform calculations including cell counting and concentration determinations, serial dilutions, and determination of antibody titer.
- Students will demonstrate technical proficiency in the following laboratory techniques: micropetting, light microscopy (including Köhler illumination adjustment), ELISA, Agglutination assays, Ouchterlony, animal injection, western blot, flow cytometry and fluorescent microscopy.