Steps for working as an undergraduate in the Weber lab.

1) Library training: An important part of research is keeping up with the current discoveries. When you start working on a project you will be expected to work to become an expert in that area. When you go to present at conferences you will need an in depth knowledge of the material. As a researcher and scientist I expect that you will regularly read scientific papers to keep up on discoveries published in the field. The BYU science librarian will train you on how to conduct literature searches for your research and how to access the online resources available at BYU. Schedule an appointment with Dr. Greg Nelson at http://guides.lib.byu.edu/mmbio and request instruction on how to do a literature search.

I have completed the library literature research component

Signed_____ Dr. Nelson's signature_____ Date_____

2) Immunology background: Immunology is a fascinating field with so many interesting aspects to learn. It will be good to sign up for the Infection and Immunity (MMBIO 261) and Immunology (MMBIO 408) courses in the future if you have not already taken them. In the mean time there are some good introductory immunology videos online that will introduce you to some of the terms and concepts you will see in the papers we read in journal club. Please go to the Khan Academy website (https://www.khanacademy.org/science/biology/immunology) and watch the 8 short video's on Immunology to get up to speed on the basics of immunology.

I have watched the 8 online Immunology video's

Signed_____ Dr. Weber's signature_____ Date_____

3) Journal Club: If you want to be able to do scientific research it requires dedication in applying yourself to learn new material and finish projects. To help with your training and to make sure you are committed to working at this we will have you participate in lab meeting and journal club for a semester where we will review how to find and read scientific papers and then you will present a paper to our research group for a number of weeks (a different paper each week). The paper must be relevant to our research, in a good journal, and current in the literature. You will be given approximately 20 minutes to present the paper by describing what was done and learned in each figure. You need to be prepared to answer questions about the paper to ensure you know what you are talking about and that you have a good understanding of the science. At the end of the presentation you should summarize what was done, what was learned, and what the next steps are. Important points to know are: What hypothesis are they testing? How did they test it and are their conclusions justified? What is the significance of the finding in the field? It is important that you attend each week and will be required to start over if do not attend.

I completed the introductory Journal Club_____ Dr. Weber's signature_____
Date _____

4. A) **Safety training:** Before you can work in a lab at BYU you must complete laboratory safety training. Proper training on safety will help prevent potential injuries or accidents. The training is completed at Y Train (http://training.byu.edu/ytrain) and involves online instruction with short quizzes. You need to complete the "**General lab safety training**" and "**Biosafety cabinet training**" modules online. You will receive confirmation that you have completed the training and must email this to your faculty mentor. It is also required that you complete **Lab specific safety training** (done by Dr. Weber).

I completed the online safety training: _____ Mentor's signature_____ Date _____

B) **Safety training renewal:** The safety training described in (1) is valid for 1 year. Thereafter, you will need to renew your safety training each Fall. This is done at the same online site and you should email proof of completion to your faculty mentor.

Date your safety training was last completed _____

C) **Mouse Safety Training:** You will need to complete five online training modules for working with mice on the BYU IACUC website (https://orca.byu.edu/iacuc/Login.php). Click the "AALAS Training" link to the right (underneath "IACUC Links"), then follow the instructions to create an account, link it to BYU, and take the proper courses. You will need to take the modules called "Maintaining Animal Procedure Areas," "Working with the BYU IACUC," "Working with the Laboratory Mouse," "Mouse Breeding Colony Management," and "**AVMA Guidelines for the Euthanasia of Animals: 2013 Edition**." To find the courses, log-in to the AALAS webpage, click "Libraries," "Animal Care and Use Courses," and use the "Course Search" tab at the bottom left-hand side of the page. There is a 25 question multiple choice quiz associated with every training module, and you will need to score above 80% to pass.

You will also need to read two PDFs from the BYU IACUC website (https://orca.byu.edu/iacuc/Login.php). Click the "Animal Care Worker Training" link to the right, and then read "Investigator General Training" and "Investigator LSB Training."

After you have completed the online readings and trainings, you can schedule your mouse facility training by contacting Warren Bingham (wwb@byu.edu or 801-857-1217) or Russ Matheson (Russm@byu.edu or 801-592-7278 cell 801-422-3979 office). Make sure to include your net ID and that you will be working in the Weber Lab. Please sign and date below after completion and report to Dr. Weber by next lab meeting as he will also need to add you to the protocol (kept with the ORCA office) before you can be granted ID card access.

I completed the online modules for working with mice_____ Dr. Weber's signature_____ Date _____

D) In the lab we use the bacteria *Listeria monocytogenes* to infect mice and test their immune responses. This is a Biosafety level 2 organism and we have a Weber lab Biosafety manual in this packet (and on the lab webpage). Please read over it and sign the last page when you are done.

I read the biosafety manual and will follow the safety rules_____ Dr. Weber's signature_____ Date _____

5) Laboratory Technique Training: Once these steps are complete you will spend a few weeks learning basic research techniques, including how to make media, pour DNA gels, run a PCR reaction, performs an ELISA, do cell culture, freeze down cells and bring them back up, accurately complete cell and viability counts and other general techniques. Once you successfully completed this stage of training you will be come involved on an ongoing project working directly with a lab instructor. This will hopefully lead to your first presentation at a major science meeting. From this you will have enough experience to work along side others and become involved in a particular aspect of the original project or perhaps start your own project.

I completed laboratory technique training_____ Dr. Weber's signature_____ Date _____

6) Consistent effort: Participation in this program should help improve your chances to obtaining top positions in graduate or professional schools. This program will help you be successful in your chosen career only if you are prepared to put in the effort. If you put in the time and effort to master the material and become proficient at evaluating science and performing laboratory work you will be able to be able to present your work at National and International conferences. This program will start the second week of the semester. You can sign up for one credit of MMBIO 494R if you need hours. As you progress into research you can sign up each semester for more hours, the maximum per semester is 3hrs. I will need your schedule to let you know a time when we can all meet each week.

Personal Page

Please complete this page as fully as possible.

Name _____ Hometown_____E-mail_____ Cell phone # _____

Year in school_____ Expected graduation date_____ Major_____ GPA_____

When did you join the lab? _____ Any previous research experience?_____

Molecular Biology or Immunology classes completed:_____

Previous biomedical training or work: _____

Bioinformatics or programming experience:_____

Language Skills_____ Interesting facts about you:_____

Project area that you are most interested in:

1. Molecular immunology:
2. Cellular immunology:
3. Other: _____

Goals in Research:_____

Goals in Career:_____

Goals in Life:_____

Picking a Journal Club Paper

Overview: Participating in a regular Journal club provides a way to keep abreast of new developments in Immunology and to learn how to read scientific papers and evaluate them. Learning how to present a journal club paper is an important skill for now and in the future.

Tips on choosing a paper: The foundation of an outstanding journal club presentation rests on the choice of an interesting and well-written paper for discussion. Pick a paper that you are interested in, that has been published in the past year, and it should usually be in one of the top journals for Immunology (see list below). While there are no guarantee that the papers in these journals are great, because of the review process that they go through to get accepted in these journals the odds are better.

Good Immunology Research Journals
Nature, Science, Cell, Nature Immunology, Immunity, Cell Host and Microbe, PNAS, PLOS Pathogens
Journal of Immunology, Molecular Immunology

Review Journals - included as a reference, we will be presenting articles from research journals.
Annual Reviews of Immunology
Nature Reviews Immunology

Keeping up on reading papers: I would recommend that you sign up to be emailed the electronic table of contents (eTOCs) for Science and Nature (register on their websites) and any other journals relevant to your work. This will help you keep up with the best new science.

Journal Impact Factors: While journal impact factors are never an indicator of how good a paper in that journal will be, if you want to get a feel for how a journal of interest is ranked here: http://admin-apps.webofknowledge.com/JCR/JCR?PointOfEntry=Home&SID=1E3@clKH8mjebkLhi18. You can search by scientific categories or search by the specific journal name.

How to read a scientific paper: Please review the basics of how to read a scientific paper at: www.sciencebuddies.org/science-fair-projects/top_science-fair_how_to_read_a_scientific_paper.shtml

Weber lab Biosafety 2 Manual (10/22/14)

One of the projects in the Weber lab involves the use of the Biosafety level 2 (BSL-2) pathogen *Listeria monocytogenes*. Everyone in the lab needs to understand the safety precautions necessary to work with *Listeria monocytogenes* and be trained on proper BSL-2 technique. Each lab member must read and then discuss this manual with Dr. Weber and get his signature as a prerequisite for working in the laboratory.

All of the BSL-2 work must be done in room 3126A LSB or the mouse room 1012B LSB. All of the BSL-2 safety policies are below, but I want to emphasize that when working with *Listeria monocytogenes* gloves and labcoats must be worn and your hands washed with soap after working with *Listeria monocytogenes* and before leaving the room. All BSL-2 samples must be processed in the hood. After use, the hood must be cleaned with a 1:10 dilution of Lysol I.C. quaternary disinfectant cleaner and 95% ethanol. Any *Listeria monocytogenes* waste samples must be place in bleach (1:10 dilution) before disposal in the biohazard containers. Flow cytometry samples need to be fixed with 2% paraformaldehyde before analysis and after analysis the tubes must be bleached and disposed of in the biohazard containers.

This manual has two parts, the 1st contains the biosafety level 2 standard practices our lab will follow (pages 6-9) and the 2nd has the Pathogen Safety Data Sheet for *Listeria monocytogenes* (pages 10-14).

Restrictions: The groups with the highest risk for *Listeria monocytogenes* infection and subsequent health risks are pregnant women, the elderly, and immune-compromised individuals. Anyone student or employee in any of these categories will not work on this *Listeria monocytogenes* project as a precaution.

I have read this biosafety manual and agree to follow the biosafety level 2 rules described.

Lab member	**Date**
Dr. Weber	**Date**

Biosafety Level 2 lab practices

Biosafety Level 2 builds upon those already used in BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

The following standard & special practices, safety equipment, and facility requirements apply to BSL-2:

A. ***Standard Microbiological Practices***

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.

2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption is not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose. Food cannot be consumed in the laboratory.

4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:

a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.

10. An effective integrated pest management program is required.

11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. ***Special Practices***

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.

3. When appropriate, a baseline serum sample should be stored.

4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.

5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.

6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.

7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.

a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.

8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.

9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.

10. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

C. ***Safety Equipment*** **(Primary Barriers and Personal Protective Equipment)**

1. Properly maintained BSCs (preferably Class II), other appropriate personal protective equipment, or other physical containment devices must be used whenever:

a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.

b. High concentrations or large volumes of infectious agents are used.
Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.

2. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.

3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.

4. Gloves must be worn to protect hands from exposure to hazardous materials.
Glove selection should be based on an appropriate risk assessment.
Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory.
In addition, BSL-2 laboratory workers should:

a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.

b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

D. ***Laboratory Facilities (Secondary Barriers)***

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.

2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.

3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.

4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

5. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.

6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.

7. Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.

8. An eyewash station must be readily available.

9. There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination.

PATHOGEN SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: *Listeria monocytogenes*.

SYNONYM: Listeria, listeriosis, listeriasis, listerellosis, and circling disease (in animals) [1, 13].

CHARACTERISTICS: *Listeria monocytogenes* is a facultatively anaerobic, gram-positive, rod-shaped coccobacillus, typically measuring 0.5 to 2μm long and 0.5μm in diameter [1 3, 4, 9]. *L. monocytogenes* has the ability to grow at low temperature, a range of pH values (between 4.3 and 9.6), and can reproduce at temperatures between 1 and 45°C [1, 3].

L. monocytogenes is divided into 11 serovars; however, most human and animal cases are caused by serovars 4b, 1/2b, and 1/2a [1, 3].

SECTION II - HAZARD IDENTIFICATION

PATHOGENICITY/TOXICITY: *L. monocytogenes* was first described as a human pathogen in the 1920s[1, 5]. Although relatively rare, human listeriosis is often severe and mortality rates can approach 50%[1, 3]. Certain factors predispose individuals to infection with *L. monocytogenes*, such as neonates, pregnancy, leukemia, Hodgkin's disease, diabetes mellitus, alcoholism or cirrhosis and immunosuppressive or cytostatic therapy[10]. Most commonly, listeria causes a mild febrile illness however, several types of listeriosis disease manifestations are recognised; for instance, listeriosis in pregnancy, listeriosis of the central nervous system (CNS), febrile gastroenteritis, glandular listeriosis, local listeriosis, typhoid listeriosis, and atypical listeriosis[10].

Listeriosis in pregnancy: Occurs mostly during the third trimester, and is characterised by a "flu like" illness with symptoms such as fever, chills, malaise, arthralgia, back pain, and diarrhoea[3, 6, 10, 12]. In many cases the infection is subclinical or inapparent; however, intrauterine infection of the foetus can lead to foetal death, spontaneous abortion, premature delivery, or the birth of a foetus that dies shortly after birth[5, 6, 10]. Surviving newborns with listeriosis are often classified as "early onset" or "late onset". Early onset neonatal listeriosis due to transplacental infection often presents as pneumonia and/or sepsis[9, 12]. Severe disease can result in widespread granulomas (granulomatosis infantisepticum)[9, 12]. Late onset neonatal listeriosis is said to occur from infection during birth, with neonates showing symptoms of meningitis one to several weeks after birth[3, 9]. In both early and late onset neonatal listeriosis, the mortality rate ranges from 20 to 30%[9].

Listeriosis of the CNS: Meningitis is the most frequently recognized listerial infection[6]. Common symptoms of listeriosis of the CNS include high fever, nuchal rigidity, tremor and/or ataxia, and seizures [6]. The most common form of non-meningitic form of CNS listeriosis is encephalitis involving the brainstem (rhombencephalitis) [6].

Febrile gastroenteritis: A non-invasive form of listeriosis that manifests as symptoms typical of gastroenteritis, for example, fever, diarrhoea, and vomiting[6, 9, 10].

Glandular listeriosis: Resembles infectious mononucleosis with swelling of the salivary glands and nuchal lymph nodes[10].

Local listeriosis: Can manifest as papules and pustules on the hands and arms following direct contact with infectious material, and can be accompanied by constitutional symptoms (fever, myalgia, and/or headache)[13].

Typhoid listeriosis: Characterised by high fever and is particularly frequent in immunocompromised individuals[10].

Atypical listeriosis: Rare cases of have been described with symptoms such as endocarditis, purulent (mononuclear) pleural exudates, pneumonia, urethritis, and abscesses[10].

EPIDEMIOLOGY: Listeriosis occurs worldwide, but is seen mostly in industrialised countries[3, 4]. Although *L. monocytogenes* was described as a human pathogen in the 1920s (mistakenly thought to be the cause of infectious mononucleosis), the first documented outbreak of food-borne listeriosis was in 1979 in 23 patients in a Boston hospital[3, 5]. The first confirmed outbreak in Canada, and first definitive link of listeriosis cases to food, was in 1981 in the Maritime Provinces and was due to consumption of contaminated cabbage in coleslaw[5]. Further outbreaks occurred during subsequent years and were often associated with a particular food type, from vegetable products in the early 1980s, to dairy products in the mid 1980s and early 1990s, to ready-to-eat meat and poultry products in the late 1990s to early 2000s[2, 5]. Indeed, ready-to-eat meat and poultry products were responsible for a multi-state outbreak in the United States in 1999 that resulted in 101 cases of illness and 21 fatalities, and more recently in Canada (originating in North York, Ontario) in August 2008, resulting in 57 confirmed cases (mostly in Ontario) and 22 confirmed deaths[2, 7].

HOST RANGE: *L. monocytogenes* has been isolated from many organisms, including humans and other mammals, fish, crustaceans, and insects[4, 10].

INFECTIOUS DOSE: The approximate infective dose of *L. monocytogenes* is estimated to be 10 to 100 million colony forming units (CFU) in healthy hosts, and only 0.1 to 10 million CFU in individuals at high risk of infection[11].

MODE OF TRANSMISSION: The predominant mode of *L. monocytogenes* transmission is by ingestion of contaminated food[1, 5, 10, 11]. *L. monocytogenes* can also be transmitted transplacentally from mother to child during pregnancy and via the birth canal during birth[3, 9, 10, 12]. Direct contact with diseased animals may lead to transmission to farmers and veterinarians during the birthing of domestic farm animals[13]. Nosocomial infections and person-to-person transmission (excluding vertical) are recognised but rare[1].

INCUBATION PERIOD: Can vary depending on the mode of transmission and dose received, but typically ranges from 1 to 4 weeks, and can be as high as several months[4, 10]. Febrile gastroenteritis as a result of *L. monocytogenes* has a short incubation period, typically 18 to 20 hours[2, 9].

COMMUNICABILITY: *L. monocytogenes* can be transmitted from mother to child during pregnancy and childbirth[3, 9, 10, 12].

SECTION III - DISSEMINATION

RESERVOIR: Soil, manure, decaying vegetable matter, silage, water, animal feed, fresh and frozen poultry, fresh and processed meats, raw milk, cheese, slaughterhouse waste, and asymptomatic human and animal carriers[4].

ZOONOSIS: Yes, through consumption of foodstuffs containing infected animal products, manure contaminated vegetables, and by direct contact with animal tissues during birthing and butchering[1, 5, 9, 10, 13].

VECTORS: None.

SECTION IV - STABILITY AND VIABILITY

DRUG SUSCEPTIBILITY: Susceptible to most broad spectrum and gram-positive spectrum antibiotics, except the cephalosporins are active against *L. monocytogenes in vitro*[8]. *In vivo*, the most active are ampicillin and amoxicillin[8].

SUSCEPTIBILITY TO DISINFECTANTS: At room temperature, *L. monocytogenes* is susceptible to sodium hypochlorite, iodophor compounds, and quaternary ammonium compounds[14]. Five to 10-fold higher concentrations of the above compounds are required at 4°C[14].

PHYSICAL INACTIVATION: *L. monocytogenes* can be inactivated by ozone, high pressure (500MPa), and high temperatures (at least 70°C for 2 minutes)[14, 15].

SURVIVAL OUTSIDE HOST: *L. monocytogenes* is commonly found in nature, particularly in association with soil, is relatively heat resistant, can tolerate cold temperature environments well, and can survive at low pH[9, 15].

SECTION V – FIRST AID / MEDICAL

SURVEILLANCE: Monitor for symptoms. Listeriosis can be diagnosed in the laboratory by cultivation of the organism, and demonstration of the infectious agent or its products in tissues or body fluids [1, 10]. Several commercially available kits exist for the detection of *L. monocytogenes*. These rapid procedures are based on ELISA and PCR technology; however, none have been validated for use as a diagnostic tool [2, 4].

FIRST AID/TREATMENT: Treatment for human listeriosis with ampicillin or amoxicillin together with gentamicin is the primary choice of therapy[8]. The recommended course of treatment is ampicillin for 2 to 4 weeks[10]. The addition of gentamicin for 2 weeks should be considered for immunocompromised patients[10]. An alternative therapy for individuals allergic to β-lactams is intravenous co-trimoxazole[10].

IMMUNIZATION: None currently available.

PROPHYLAXIS: No chemoprophylaxis exists; however, precautions for individuals who are immunocompromised or pregnant women include the avoidance of raw food and vegetables, undercooked meat, soft cheeses, and cheeses prepared from unpasteurised milk[10].

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: A particularly rare laboratory-acquired infection with some suspected cases, none of which have been confirmed[16].

SOURCES/SPECIMENS: Blood, cerebrospinal fluid, faeces, placenta, skin lesions, pus, amniotic fluid, menstrual blood, lochia, respiratory secretions, meconium, gastric aspirate, animal tissues/specimens, and infected organs such as brain and liver[2, 4, 10].

PRIMARY HAZARDS: Accidental autoinoculation, exposure to the tissues of experimentally infected animals, and *L. monocytogenes* cultures[17].

SPECIAL HAZARDS: Those at greater risk of infection (pregnant women or immunocompromised individuals) should take extra care when working in a laboratory where *L. monocytogenes* is propagated or handled[3].

SECTION VII – EXPOSURE CONTROLS / PERSONAL PROTECTIONRISK GROUP CLASSIFICATION: Risk Group 2[18].

CONTAINMENT REQUIREMENTS: Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious material, animals, or cultures.

PROTECTIVE CLOTHING: Lab coat. Gloves when direct skin contact with infected materials or animals is unavoidable[17]. Eye protection must be used where there is a known or potential risk of exposure to splashes.

OTHER PRECAUTIONS: All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC)[17]. The use of needles, syringes, and other sharp objects should be strictly limited. Additional precautions should be considered with work involving animals or large scale activities.

SECTION VIII – HANDLING AND STORAGE

SPILLS: Allow aerosols to settle and, wearing protective clothing, gently cover spill with paper towels and apply an appropriate disinfectant, starting at the perimeter and working towards the center. Allow sufficient contact time before clean up[17].

DISPOSAL: Decontaminate all wastes that contain or have come in contact with the infectious organism before disposing by autoclave, chemical disinfection, gamma irradiation, or incineration[17].

STORAGE: The infectious agent should be stored in leak-proof containers appropriately labeled[17].

SECTION IX - REGULATORY AND OTHER INFORMATION

UPDATED: December 2011

PREPARED BY: Pathogen Regulation Directorate, Public Health Agency of Canada.

Although the information, opinions and recommendations contained in this Pathogen Safety Data Sheet are compiled from sources believed to be reliable, we accept no responsibility for the accuracy, sufficiency, or reliability or for any loss or injury resulting from the use of the information. Newly discovered hazards are frequent and this information may not be completely up to date.

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Weber Lab Techniques Introduction

In order to be useful and productive in the lab we have put together this manual that you will be required to complete and return to Dr. Weber. By completing these tasks you will be prepared to successfully do research in the lab.

Each sheet has requirements that you need to have passed off by lab members to signal that you are considered competent to a particular task. Do not feel embarrassed to ask to be shown some of the task we would rather that you learn correctly first time. Please take this seriously, it will save you and the lab time and money. We expect you to make and keep really good lab notes on all that you are shown so that you will not need further instruction on any techniques you have passed off. Make sure you understand the background to what you are doing and ask all the questions you can think of when a technique is first demonstrated.

You should keep very detailed notes in a proper notebook that should sustain you if you ever require a technique when it is late at night and there is no one around. If you are not maintaining appropriate effort you may be asked to consider moving to a different lab. **By the time you have finished each technique you should have mastered it well enough to correctly teach others.**

For most techniques it will require current labbies to sign off that they agree you have competed the task.

Your Name _____

Email_____

Contact Phone number_____

Lab Notebook Entries

Page #
Date
Student name
Experiment name

A. Purpose of Procedure

This section is very important. This is not a place to write a one-sentence description of the protocol you are going to do *this* moment, but a place to describe how the protocol fits in to the broader purpose of what is to be accomplished and why. Think about what finding caused you do this experiment, what you want to determine, and what is to be done next.

B. Hypothesis and expected results

What is your hypothesis? Describe the protocol you will be using to test this hypothesis. If everything goes as hypothesized, what will happen?

C. Actual Protocol

Describe the protocol as it is *actually* done. What is actually done may or may not be different from the theoretical protocol… just make sure that a note of everything that is different is made. This is important for any future references made back to this notebook entry for "tips" on how to (or *not* to) do something. There may be information that you currently do not think is important that may be important after additional experiments.

D. Results

The data/results that you actually got . . .no matter how exciting or crazy. Numbers and calculations go in this section. Once again, there are many details that seem clear now that you may forget, write them down now.

E. Conclusions

How well do the Experimental Results match up with the Expected Results. The most important part of this section is what is written when the Experimental Results *don't* match up with the Expected Results; it is under these circumstances that thoughts about why there is a discrepancy are critical.

Proper Notebook Keeping

1. Your notebook is the property of the BYU. It should never leave the lab. It should have on the front cover your name, Dr Weber's name, and the lab room and phone number (422 6716) and a statement saying if found please return to Dr Weber 3137 LSB Brigham Young University, (422 6259) as it contains very important research data.
2. Notebooks should be kept in English.
3. Every entry should have a clear date, including year.
4. Always list catalog number and lot number of reagents.
5. Always write stock and final concentration of reagents.
6. Always write down how a reagent is made, and label the reagent with the page reference in your notebook, so that someone who picks up a tube can track what's in it. Also, if one needs to make up fresh reagent it can be repeated reproducibly.
7. Every experiment should have goal or hypothesis information and proper controls where possible positive and negative controls.
8. Always write step-by-step protocol. If you are following a standard protocol reference the page number in your notebook where the protocol is written out, and clearly describe any deviations from the protocol. If you are following a manufacturer's protocol, specify this in your notebook, with manufacturer's document number (if there is one).
9. Someone who picks up your notebook a year from now should be able to easily reproduce what you did without having to ask about it.

I have been instructed on how to keep a lab notebook. This needs to be done in an organized manner that will allow someone else to find the experiments you did and replicate them if needed.

Sign _____ Super _____ Date _____

I have been instructed on how to store data on the lab computer. All data needs to be accessible on the lab computer and not stored on personal laptops or computers.

Sign _____ Super _____ Date _____

I have been instructed on what to do if the CO_2 is low and the alarm goes off (switch tanks, call Dr. Weber if needed).

Sign _____ Super _____ Date _____

I have been instructed on making sure that the -80°C freezer is properly closed.

Sign _____ Super _____ Date _____

I have been instructed what to do in the event of an emergency.

Sign _____ Super _____ Date _____

I have been instructed on how to obtain ice for laboratory use.

Sign _____ Super _____ Date _____

I have been instructed on how to obtain filtered ddH_20 for laboratory use.

Sign _____ Super _____ Date _____

I have been instructed on how to make up PBS. The formula for PBS is as follows:

Sign _____ Super _____ Date _____

I have been instructed on how to aliquot reagents to prevent continual freezing and thawing

Sign _____ Super _____ Date _____

I have been instructed on how to make up difficult to dissolve solutions.

Sign _____ Super _____ Date _____

I have been instructed in how to sterile filter small quantities of liquid.
Describe:

Sign _____ Super _____ Date _____

I have been instructed in how to sterile filter large quantities of liquid.
Describe:

Sign _____ Super _____ Date _____

I have been instructed on how to look up protocols on the lab computer and online.

Sign _____ Super _____ Date _____

I have been instructed on how to look for basic lab recipes

Sign _____ Super _____ Date _____

I have been instructed on how to look up details of cell lines from ATCC

Sign _____ Super _____ Date _____
I understand before I work with animals I have to obtain IACUC approval and also complete appropriate animal courses.

Sign _____ Super _____ Date _____

I understand before I work with any chemical that I am expected to read and ensure the appropriate MSDS is in the lab folder. I will comply with all the safety statements on the MSDS sheet.

Sign _____ Super _____ Date _____

I understand that if I require after hours access to the lab (late at night, over weekends and on holidays when the building is closed) I need to obtain the appropriate approval.

Sign _____ Super _____ Date _____

I understand that if a situation ever arises that I do not have the training to deal with, I am responsible to contact someone who can take care of the problem.
I will contact Super _____(Phone number) _____in the event that such a situation arises.

Sign _____ Date _____

Note: I understand that, while the social aspects of the laboratory are great and welcome, the main purpose of the laboratory is to do great research. Computers, equipment, and space should go first and foremost to people actively doing research.

Sign _____ Date_____

I realize that it is my responsibility if I am the last person to leave the lab no matter what time of day to ensure:

1. The Bunsen burners are switched off
2. The $C0_2$ tanks have sufficient supply
3. The -80o C Freezer is closed properly
4. The lab is cleaner than when I arrived.
5. The biohazard bag is not overflowing
6. Experimental reagents are put away
7. My lab notebook is updated and put away.

Sign _____ Date _____

Waste Disposal

1. Regular trash: all paper, cardboard, and plastics that are uncontaminated. Janitorial staff empties these daily.
2. Red biohazard bags: all solid biohazard waste. When full, tie them closed call Chemical Management at 422-6156 or http://chemmgmt.byu.edu/ (Contact Dr. Weber for website access)
3. Sharps containers: all needles, blades, broken glass.
4. Radioactive waste: We currently do not use radioactive materials, if needed discuss this with Dr. Weber
5. Chemical waste: Chemical waste goes into segregated containers. When full, call Chemical Management (422-6156 or http://chemmgmt.byu.edu/)
6. Animal waste: Carcasses get placed in biohazard bags and frozen until removed by chemical management.
7. Sink disposal: the only things that can go down the sinks are aqueous buffers. Do NOT dispose of methanol down the sinks!!

I have read and agree to abide by the above approved waste disposal methods.

Sign _____ Date_____

Making and Diluting Solutions

General information on diluting and making solutions.

A few general precautions and important points

1. When making solutions from powders, add to less than total volume and bring up to correct volume when dissolved.
2. Know your reagents! Check that the lab has a current MSDS for each chemical that you are working with if it does not then obtain one before beginning the research with the chemical. Know if they are dangerous, and what protective steps to take!
3. When diluting acid, add acid to water (NOT the other way around). This is exothermic. Add it slowly. Work in the chemical hood.
4. Dissolving a base in water is very EXOTHERMIC. Expect this! Add it slowly, pre-chill the water if necessary, make the solution on ice if necessary. For example, if I make a 10M solution of NaOH, I generally add the pellets in 1M increments, and chill the solution in between. Work in the chemical hood.
5. Viscous solutions (for example Tween, Triton). You cannot accurately pipette a viscous solution. Use a syringe. If you need a very small amount (microliters), consider making an intermediate solution (say, 10%), which you can then use to make your final solution.
6. If you are using an antibody, keep in mind that the dilution a protocol lists is for a specific antibody from a specific company. Check the catalog number to make sure. A different antibody, even if it is against the same antigen, will probably have a different needed dilution.

Making and Diluting Solutions

1. Molarity. Molarity is moles per liter. The molecular weight of a substance is the number of grams one mole of that substance weighs. Therefore, the molecular weight in grams in one liter equals a 1M solution. Here is a website on molarity:http://environmentalchemistry.com/yogi/chemistry/MolarityMolalityNormality.html
    a. Molality: moles/kg solution; Normality: Molarity x n, where n=# of protons exchanged in the reaction
2. Fold solutions (10x etc). A 10x solution is 10 times the final concentration. Note that this is specific to an individual application. It works well for standard buffers like PBS or TBS or TBST. But, if you make a 10x solution for one protocol, don't assume it is the correct concentration for another protocol without checking actual initial and final concentrations.
3. Percent solutions
    a. Liquids. % equals ml of solute per 100ml of solvent.
    b. Solids. % equals gm of solute per 100ml of solvent.
    c. NOTE: Be sure what form your chemical exists in out of solution. In general, most salts and bases exist as powders, and most acids, organics, alcohols, biologicals exist as fluids.
4. Diluting in moles or percents. Very useful equation: Vs x Cs = Vf x Cf. Vs is Volume stock, Cs is concentration stock, Vf is volume final, Cs is concentraton final. Solve for the one you don't know.
    a. **Dilutions.** Mostly for things like antibodies. Technically 1:100 means 1 unit solute plus 100 units diluent, whereas 1/100 means 1 unit solute per 100 unit final solution (so 1 unit solute plus 99 units diluent). In general we use 1:100 type terminology but really mean 1/100. This doesn't matter much when diluting a lot, but is very important when diluting something like 1/2 (which is 1:1)—so 1 unit of solute plus 1 unit diluent.
    b. **Cell culture splitting.** In cell culture the meaning is slightly different: a 1:10 split is one tenth of the original cells into the new flask, independent of how much media you add. Thus, trypsinizing cells, re-suspending in 10 ml media, adding 1ml to a new flask plus 9 ml media is a 1:10 split. However, re-suspending in 5 ml, adding 0.5ml cells plus 4.5 ml media is ALSO a 1:10 split.
    c. **Serial Dilutions.** Serial dilutions mean each dilution is made from the previously diluted solution. This is necessary when doing very dilute solutions because pipettes are simply not accurate for extremely low volumes. Often (but not always), serial dilutions are the same ratio for each step (e.g. 1/2, 1/4, 1/8, 1/16 etc).

Examples

1. Calculate how much NaCl you need to make 100 ml of a 0.5M solution of NaCl. MW=58.44.
    a. 58.44g/L=1M
    b. You want 0.5M, so multiply the MW by 0.5:
    c. 29.22g/L=0.5M
    d. You want 100ml which is 0.1L so multiply the MW by 0.1:
    e. 2.922g/100ml=0.5M