SAVE THE BABIES!

A Lab Based Curriculum
Focused on Necrotizing Enterocolitis, NEC
NIH Curriculum: Save the Babies! – Lab Based Curriculum Focused on Necrotizing Enterocolitis, NEC
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Special Thanks to:
Josef Neu, M.D., Nan Li, M.D. - Shands Hospital, University of Florida, Department of Pediatrics

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The content is solely the responsibility of the author and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health.

Additional information regarding the Bench to Bedside project is available at http://www.cpet.ufl.edu/bench.

Please direct inquiries to the Center for Precollegiate Education and Training at cpet@cpet.ufl.edu.

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Author’s Note

This unit is designed for any class that teaches the ExD in detail, but it is my intention to use this unit to help AICE Biology students pass the A Level Exam as this unit will help them with Paper 5.

However, for the purpose of focusing on the AICE curriculum, all assessments will be judged along the guidelines of Teaching A Level Biology Skills. In the course of these investigations, it should be noted that the students may not all have the exact same results as the other benches. This is intentional as it is often within the realm of scientific inquiry that different researchers obtain different results and the never ending quest continues. There are some labs where a range may be indicated and the students will make judgment calls. If their call supports their research, then that should count as conclusive material.

The students will be called upon to create a Plan Diagram in lesson one. A plan diagram is the students own drawing of their interpretation of where tissue differentiation occurs or a drawing of a grouping of cells. The specifications for the drawings are not open to interpretation. For example, if one is drawing a cell wall, then the line depicting that must be continuous and unbroken. If a cell wall were broken, it would indicate death of that cell. Complete instructions on how to make a plan diagram, including a link to a youtube video are included in the appendix.

The last two assignments are higher order critical thinking skills of analyzing and evaluation. According to the AICE curriculum, an analysis will include a description and summary of the results, including any perceived errors or outliers. An evaluation is to judge the effectiveness of the procedures, if the errors could be due to equipment failure and if so be able to suggest modifications. A complete summary of the 7 skills is found in the appendix.

One may also be searching for and not finding any student work sheets in this unit. The A Level AICE Biology is heavily dependent upon original student submission, written in their prose. This includes all the steps of the ExD including the results and conclusion. Therefore, one will find that the students are to submit their work via their scientific notebook. One highly recommended notebook is the Hayden-McNeil Scientific notebook with carbon copies. When the students are to hand in their work, they will keep the original and turn in a carbon copy. This is a close approximation of what one would find in an authentic research facility. A power point explaining the benefits of this notebook are also included in the appendix.
Introduction

This unit focuses on a devastating disorder that affects 7-10% of pre-term neonates, Necrotizing Enterocolitis or NEC. This disorder is devastating to the parents and fatal in 20-30% of the cases. The summer of 2012 I had the privilege of observing the work of Josef Neu, M.D. and his lab director Nan Li and reviewed their research and present work in preventing and caring for these afflicted babies. I developed the following unit based upon their work at the University of Florida, Shands Hospital, in hopes that the students would grasp the complexity and also the uncertainty of research work.

Research work follows the same protocol whether a researcher is in a primary grade school on up to post doctorate level. There is a global understanding that the scientific method be followed and adhered to regardless of the country or culture. There are logical steps that are followed, some simple rules and honesty and integrity when it comes to reporting findings. In any science curriculum it is paramount that a researcher understands this universal protocol. This unit is geared toward the high school level of comprehension for the application of the scientific method. This paper will refer to this method as the experimental design, or ExD. NEC was chosen as the disorder of research due to the high level of emotionality involved.

The rationale for choosing the ExD as a focus for comprehending this unit is a result of reading America’s Lab Report (National Academy of Sciences 2006), adherence to the Next Generation Sunshine State Standards and the University of Cambridge AICE biology Curriculum guides.

According to America’s Lab Report, the quality of current laboratory experiences is poor for most students in America. They have found that teachers and lab manuals often emphasize procedures over learning goals and that most high school lab experiences do not follow the instructional design principles for effectiveness identified by the National Academy of Sciences. Unfortunately it is true, what I also see is that in most high school science classes, labs are focused on procedures and results, usually a cookie cut hand out. Students think they are creating labs without looking at the entire ExD which includes initial observations and questions. Even more important, many of the labs found in typical science lab books often do not follow up on how to correctly post results, what to do if there are perceived errors, and how to write a conclusion that will result in further research.

America’s Lab Report focuses on four principles of instructional design that can help lab experiences achieve their intended learning goals. They are:
1. The labs are designed with clear learning outcomes in mind.
2. They are thoughtfully sequenced into the flow of classroom science instruction.
3. They are designed to integrate learning of science content with learning about the process of science.

The Next Generation Sunshine State Standards for the state of Florida, Standard 1: The Practice of Science also addresses the issue of successfully including the ExD in a curriculum. This standard reads, SC.912.N.1.1: Define a problem based on a specific body of knowledge, for example: biology, chemistry, physics, and earth/space science, and do the following:
1. Pose questions about the natural world,
2. Conduct systematic observations,
3. Examine books and other sources of information to see what is already known,
4. Review what is known in light of empirical evidence,
5. Plan investigations,
6. Use tools to gather, analyze, and interpret data (this includes the use of measurement in metric and other systems, and also the generation and interpretation of graphical representations of data, including data tables and graphs),
7. Pose answers, explanations, or descriptions of events,
8. Generate explanations that explicate or describe natural phenomena (inferences),
9. Use appropriate evidence and reasoning to justify these explanations to others,
10. Communicate results of scientific investigations, and
11. Evaluate the merits of the explanations produced by others.

According to the Cambridge International A & AS Level Biology Syllabus, in the supporting material Teaching A Level Biology Practical Skills, there are 7 practical skills that contribute to the overall understanding of scientific methodology. In a scientific investigation these would be applied in the following sequence:

I. Planning the experiment
II. Setting up/manipulating apparatus
III. Making measurements and observations
IV. Recording and presenting observations and data
V. Analyzing data and drawing conclusions
VI. Evaluating procedures
VII. Evaluating conclusions

Since this unit is focused primarily on bench and lab work, the implementation of the experimental design template in this unit is paramount. The experimental design, is a calculated procedure of focusing on one independent variable at a time to determine any measurable effects on the dependent variable. In this unit, we want to save the babies after having identified the bio marker in autopsies. Therefore, three out of 6 of these lessons will be approached and assessed using the ExD. The template for following the ExD is found in the appendix of this unit.

This unit begins with Dr. Neu’s paper on Necrotizing Enterocolitis and some avenues of research that may possibly make identifying the high risk babies easier and treatment a more viable option. The three labs included in this unit focus on: determining the bio-marker for NEC, earlier detection with a less invasive technique for detection of the bio–marker and a lab that focuses on how to properly dose an afflicted baby so that treatment is meaningful and effective.
Tips about this Curriculum

**Lesson Plan Format:** All lessons in this curriculum unit are formatted in the same manner. In each lesson you will find the following components:

- **KEY QUESTION(S):** Identifies key questions the lesson will explore.
- **OVERALL TIME ESTIMATE:** Indicates total amount of time needed for the lesson, including advanced preparation.
- **LEARNING STYLES:** Visual, auditory, and/or kinesthetic.
- **VOCABULARY:** Lists key vocabulary terms used and defined in the lesson. Also collected in master vocabulary list.
- **LESSON SUMMARY:** Provides a 1-2 sentence summary of what the lesson will cover and how this content will be covered. Also collected in one list.
- **STUDENT LEARNING OBJECTIVES:** Focuses on what students will know, feel, or be able to do at the conclusion of the lesson.
- **STANDARDS:** Specific state benchmarks addressed in the lesson. Also collected in one list.
- **MATERIALS:** Items needed to complete the lesson. Number required for different types of grouping formats (Per class, Per group of 3-4 students, Per pair, Per student) is also indicated.
- **BACKGROUND INFORMATION:** Provides accurate, up-to-date information from reliable sources about the lesson topic.
- **ADVANCE PREPARATION:** This section explains what needs to be done to get ready for the lesson.
- **PROCEDURE WITH TIME ESTIMATES:** The procedure details the steps of implementation with suggested time estimates. The times will likely vary depending on the class.
- **ASSESSMENT SUGGESTIONS:** Formative assessment suggestions have been given. Additionally, there is a brief summative assessment (pre/post test) that can be given. Teachers should feel free to create additional formative and summative assessment pieces.
- **EXTENSIONS:** (ACTIVITIES/LITERATURE) There are many activities and reading sources available to augment and enhance the curriculum. They have been included. If you find additional ones that should be added, please let us know.
- **RESOURCES/REFERENCES:** This curriculum is based heavily on primary sources. As resources and references have been used in a lesson, their complete citation is included as well as a web link if available. All references and resources are also collected in one list.
- **STUDENT PAGES:** Worksheets and handouts to be copied and distributed to the students.
- **TEACHER PAGES:** Versions of the student pages with answers or the activity materials for preparation.

**Science Subject:** AICE Biology

**Grade and ability level:** 11th and 12th grade students enrolled in the AICE Biology class

**Science concepts:**
Lesson Summaries

LESSON ONE: Saving babies! A Review of the Scientific Literature Regarding Necrotizing Enterocolitis (NEC)
- Power point presentation that will review the article and help with comprehension of the biomarker of interest, Interleukin 1 receptor antagonist, IL-1ra.
- Preview of the 3 bench labs and the 2 papers to conclude this unit.

LESSON TWO: Why did my baby die?
- Proteomics wet lab to look for presence of absence of IL-1ra. An inquiry lab designed to look for a biomarker molecule, one to be a defining feature of NEC.

LESSON THREE: Is my baby going to be alright?
- Wet lab Dot Blot technique. An inquiry based lab to determine if the presence or absence of IL-1ra is a predictor of risk assessment in NEC.

LESSON FOUR: Save my baby!
- Wet lab serial dilution to determine medicinal dosing of probiotics. An inquiry based lab designed to give the students a range of options and make a judgment call on dosing.

LESSON FIVE: Results Paper
- Students will be aggregating the data from all the different lab benches and offering an analysis and evaluation of the data.

LESSON SIX: Conclusion Paper
- Students will be writing a conclusive paper in the role of either an M.D. breaking the news to the parents of a baby that has just been diagnosed with NEC, or a PhD pleading for other benches to join the fight and help save the babies.
Lesson Sequencing Guide
Vocabulary

500g, 1000g, 1500g converted to pounds: 500g = 1.1lbs, 1000g = 2.2lbs, 1500g = 3.3lbs

Antibody: a Y shaped protein produced by B-cells that identify and neutralize antigen molecules

Antibody-antigen complex: a known coupling of an antigen with an antibody. These are highly specific and usually related in terms of the immune response. An antigen would be a foreign or non-self molecule and the leukocytes will attach a matching/fitting molecule to disable the antigen from doing any harm.

Antigen: a molecule or cell fragment that provokes the immune response. This response will often include swelling of tissue and the production of antibodies. Self-antigens are tolerated by the immune system, non-self will be attacked by the immune system.

Anti-inflammatory:

Buccal swabs: by swabbing with a cotton swab on the inside of the cheek to obtain a cell sample

Commensal bacteria: beneficial bacteria to the gut. Exact and entire role for human and animal health is not fully understood.

Conclusions: after an analysis of the data, one draws a conclusion by summarizing the key points and presenting them in a clear coherent style. A good conclusion will also include an evaluation of the data, taking into account possible errors and the effects they would have on data.

Dosing:

Dot blot: a diagnostic test to determine if a molecule of interest is present in a tissue sample. The molecule must have a known antibody.

Gel Electrophoresis: the lab process of separating molecules of interest from a tissue sample. The molecules are DNA, RNA or proteins.

Hypothesis: a proposal intended to explain certain facts or observations. Testable or verifiable statement.

Interleukin 1 receptor antagonist, or IL-1ra: the molecule of interest for this research. A transmembrane protein blocker involved in reducing the swelling initiated by the immune response of the IL-1 and the IL-1 receptor.

Leaky gut: disruption of the tight junctions that lead to hyper-permeability, in NEC it leads to bowel hyper-permeability

Microbiota: refers to the commensal bacteria of the gut and digestive system

Necrotizing enterocolitis: A spectrum of intestinal conditions that differ with respect to pathogenesis and strategies for prevention and treatment. Signs and symptoms include feeding intolerance, abdominal distention and bloody stools. X-rays will show a paucity of gas, gas filled loops of bowel and dilated loops of bowel. Rate of infection is 7% around low birth weight infants with a 20-30% mortality rate.

Neonate: A baby from birth to four weeks

Nitrocellulose membranes: a lab grade paper with a sticky side to immobilize nucleic acids or proteins
Primary antibody: molecules that recognize and bind to a specific protein, they are used to target biological molecules of interest

Probiotic:

Proteomics: large scale study of proteins, their structure and function.

Results:

Secondary antibody: molecules that will bind to a specific primary antibody, these will have a florescent tag associated with them. When a UV light is shown on the secondary antibody with a florescent tag, then they will glow.

Serial dilution:

Supernatant: the usually clear liquid lying above a solid residue after crystallization, precipitation, centrifugation or other processes.

Tight junctions: adjacent cells that have intercellular trans-membranes that hold or ‘stitch’ cells tight against each other. Acts as a barrier to differentiate tissues or organs.
Next Generation Sunshine State Standards – Science

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## Advanced International Certificate of Education (AICE) Biology Learning Outcomes

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LESSON ONE: Saving babies! A Review of the Scientific Literature Regarding Necrotizing Enterocolitis (NEC)

KEY QUESTION(S): The key questions that the students are going to investigate are, “What is NEC? What seems to be the cause of this disorder? Why do only some babies develop NEC and others do not? Can we help them?”

KEY SCIENCE TOPICS:
1. Reviewing scientific literature regarding the definition of NEC.
2. They will be able to describe the pathophysiology of NEC and how it creates a leaky gut. The pathophysiology will include:
   a. Altered intestinal microbiota
   b. Inadequate intestinal barrier function
   c. Excessive inflammatory response due to the immune system response and lack of IL-1ra to inhibit the swelling

OVERALL TIME ESTIMATE: One 48 minute class period

LEARNING STYLES: Visual, auditory and prose.

VOCABULARY:
Neonate: A baby from birth to four weeks

Necrotizing enterocolitis: A spectrum of intestinal conditions that differ with respect to pathogenesis and strategies for prevention and treatment. Signs and symptoms include feeding intolerance, abdominal distention and bloody stools. X-rays will show a paucity of gas, gas filled loops of bowel and dilated loops of bowel. Rate of infection is 7% around low birth weight infants with a 20-30% mortality rate.

500g, 1000g, 1500g converted to pounds: 500g = 1.1lbs, 1000g = 2.2lbs, 1500g = 3.3lbs

Microbiota: refers to the commensal bacteria of the gut and digestive system

Commensal bacteria: beneficial bacteria to the gut. Exact and entire role for human and animal health is not fully understood.

Tight junctions: adjacent cells that have intercellular trans-membranes that hold or ‘stitch’ cells tight against each other. Acts as a barrier to differentiate tissues or organs.

Leaky gut: disruption of the tight junctions that lead to hyper-permeability, in NEC it leads to bowel hyper-permeability

Antigen: a molecule or cell fragment that provokes the immune response. This response will often include swelling of tissue and the production of antibodies. Self-antigens are tolerated by the immune system, non-self will be attacked by the immune system.

Antibody: Y shaped protein produced by B-cells that identify and neutralize antigen molecules

Interleukin 1 receptor antagonist, or IL-1ra: the molecule of interest for this research. A transmembrane protein blocker involved in reducing the swelling initiated by the immune response of the IL-1 and the IL-1 receptor.

LESSON SUMMARY: For an introductory lesson NEC, the three lab assignments, and the concluding papers, it is essential that the original work of Dr. Neu and Dr. Walker be presented. First the original work with main points highlighted will be presented, then a more comprehensive style on a power point for reinforcement of learning.
While the students are engaged in a teacher led discussion on both the original paper and power point, they should be defining the vocabulary words to the best of their ability using only the contextual clues given in the two works. The students will also be assigned to create a plan diagram of tight junctions of columnar epithelial cells and what they predict a leaky gut of columnar epithelial cells will look like. At the conclusion of class, they will also have created two inquiry questions regarding any aspect of NEC.

**STUDENT LEARNING OUTCOMES**
The student will be able to...

1. Describe and interpret drawings and photographs of typical animal cells, as seen using an electron microscope. Focus on cell membrane and cell junctions.
2. Explain the meaning of the term immune response, making reference to the terms antigen, self and non-self.
3. Understand how cell signaling plays a role during the immune response.

**STANDARDS**

**PRIOR KNOWLEDGE:** Prior to this lesson, the students will have to have the discipline to read and take notes on a higher level reading comprehension. They should be encouraged not to copy verbatim, but to create short hand notes and rely on contextual clues for defining the vocabulary. Students also must have the skill set to draw a plan diagram.

**MATERIALS:**

- **ESSENTIAL:**
  - Highlighted copy of Necrotizing Enterocolitis by Josef Neu M.D. and W. Allan Walker M.D., preferably electronic
  - Power Point presentation of NEC
  - Vocabulary words
  - Plan diagram drawing skills

**BACKGROUND INFORMATION: Necrotizing Enterocolitis, or NEC,** is among the most common and devastating diseases among neonates, or newborn infants. It has been difficult to predict which babies may become infected and how they will respond to treatment, preventative, surgical or medicinal. The mean prevalence for this disorder is about 7% among infants with a birth weight between 500 and 1500 grams. Among those, there is a 20-30% mortality rate. Babies recovering from NEC have a 25% chance of microcephaly and serious neuro-developmental delays. The costs in the US are between $500 million and $1 billion due to the extended hospitalization and surgical costs. The cost for one child requiring bowel reconstruction will be $1.5 million for the child's first 5 years of life. With all of this in mind, research into this disorder in now a high priority in the medical community.

NEC is an excessive inflammatory response of the neonate’s small intestine. The initial signs include feeding intolerance, abdominal distention and bloody stools after 8-10 days of age. Abdominal x-rays show gas in the intestinal wall and/or portal vein gas. The term *necrotizing* means tissue death or tissue that is in the process of dying. The term *entero* means within and *colitis* refers to a swollen colon. Therefore, NEC is the process by which a neonate has a condition in which part of their colon is swollen, allowing for intestinal *microbiota* to leak into the surrounding tissue and is causing tissue death. Dead tissue within a living being must be removed before even more severe infections start to happen such as sepsis.

Inside the intestinal tissue is a host of *commensal bacteria.* These are good bacteria that must be allowed to thrive in our gut for health reasons. When a baby is born, the gut is sterile and they receive their first commensal bacteria through a vaginal birth, then most ideally through the handling and feeding by the mother. A baby that is successfully breast fed will receive its first passive immunity. *Antibodies* from the mother’s milk will be directly transferred into the gut of the baby. This should be the beginning of the building of a healthy gut microbiota for the baby. If a baby does not have a strong set of antibodies, then any *antigen* can spark off an inflammatory immune response. An antigen is something the body usually does not recognize, so the body goes into a defensive immune response. Immune responses usually set off an inflammatory response that allows for greater ease of
white blood cells to target the unwanted pathogens. After an immune response has been triggered, the swollen tissue needs to normalize and the swelling reduced. This is the difference between tight junctions and a leaky gut. Tight junctions are when healthy epithelial cells are held together close by transmembrane proteins and bacteria and antigens cannot cross the epithelial cells into surrounding tissue. With NEC babies, when their immune response first triggers the swelling of a tissue, the mechanism does not exist to turn the swelling off. This results in the commensal bacteria from the intestines to seep into the surrounding tissue, hence the name leaky gut. The mechanism to turn the swelling off to normalize the tissue is being hypothesized of that being Interleukin 1 receptor antagonist, or IL-1ra.

Therefore, three labs in this unit will focus on the IL-1ra molecule and the part it plays in identifying the high risk babies and diagnostic properties. The following labs will include: one to confirm the presence or absence of the IL-1ra in NEC babies, one to look for bio-markers to identify high risk babies, and one to treat NEC babies.

ADVANCE PREPARATION:

1. Electronic copy of Necrotizing Enterocolitis by Josef Neu, M.D. and W. Allan Walker, M.D., New England Journal of Medicine, 2011. This article has been highlighted for ease of comprehension. Teacher led discussion through the article as vocabulary is pointed out (attachment)
2. Power point notes to help review the article for comprehensive purposes (attachment)
3. Student assignment written on board: “In your notebook, write down the following vocabulary words. Define them during the class time using contextual clues from the article and power point discussion only:
   • Neonate, Necrotizing Enterocolitis, microbiota, commensal bacteria, tight junctions, leaky gut”
   “Draw two plan diagrams of columnar epithelial cells; one with tight junctions showing intact transmembrane proteins and a leaky gut with broken trans-membrane proteins. Plan diagrams consist of unbroken lines; there should be not shading or coloring. Lines should delineate cell membranes, a nucleus, and trans-membrane proteins. All cell should be columnar epithelial with mucosal cells interspersed. Create a brush border on the superior surface of non mucosal cells.”
   “Create two inquiry based questions from this lesson.”

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. As students enter the room, they will see the assignment on the board: the vocab, plan diagrams and creation of inquiry based questions.

2. (5 minutes) Students will write the vocabulary words on the left column of their science notebook, leaving just two rows for defining. (5 minutes)

3. (15 minutes) Open up the document Necrotizing Enterocolitis by Josef Nue, M.D. and W. Allan Walker, M.D. from the New England Journal of Medicine. There will be parts of this article that are highlighted from each section. These highlighted areas will point out the main parts of the article. Teacher led discussion through the article, careful to point out that it is a work in progress; there are no definitive conclusions to the present inquiry, just strong evidence.

4. (15 minutes) Reinforce the article with the power point. The power point starts with a broad view of microbiota and commensal bacteria. The power point then describes NEC showing the difference between the tight junctions and leaky gut. It then continues on briefly introducing the bio-marker of interest for the 3 labs and 2 papers that will complete this unit.
   a. Lab 1: Verifying that babies that had died from NEC were lacking the IL-1ra with gel electrophoresis.
   b. Lab 2: Using a Dot Blot test to determine what premature babies in the NICU are at high risk for developing NEC.
   c. Lab 3: Using a serial dilution lab to determine the correct dose of probiotics and medicine for different birth weight babies.
5. Students will turn in their papers with the vocabulary, two plan diagrams and 2 inquiry based questions upon class dismissal. The slip is confined to one page from their notebook. Remaining class time until dismissal. Students not finished with assignment may turn it in the next day.

ASSESSMENT SUGGESTIONS

- The following format will be posted on the board for the students to follow. They will turn in one carbon copy from their notebook at class dismissal or the next day if they do not finish in class:
  - **Vocabulary**: Copy the vocabulary words from the board on the left side of your lab notebook. Allow for 2 lines. Define the words on the right using only today’s information and your existing schema of knowledge. Do not look up the words using other resources.
  - **Draw a plan diagram of healthy intestinal epithelial columnar cells and a leaky gut. Label the following:**
    - Nucleus
    - Tight junctions
    - Brush border
    - Mucus
    - Commensal bacteria
    - Leaky gut with inflammation
  - **Create two questions you may ask as a researcher.**

- Objective 1 is covered on the labeled plan diagram.
- Objective 2 is covered on the vocabulary portion.

- This lesson will be graded for completion, 10 points per Roman numeral. The justification for this grading system is to create analytical thinking skills and not be judged on correctness (except for plan diagram, which must be correctly labeled).

EXTENSIONS:

ACTIVITIES:

- This unit will continue on with 3 different biotech labs, and a results and conclusion paper. See lessons 5 and 6 for a more extensive explanation of a results and conclusion paper. These papers will be written from the perspective of either an M.D. or a PhD medical researcher. An M.D. will present data to parents, the PhD to other researchers looking for extensions.

RESOURCES/REFERENCES:

LESSON TWO: Why Did My Baby Die?

KEY QUESTION(S): When parents are faced with the unexpected infection and consequent death of a neonate, their first question is often, "Why? Why did my baby get sick and die?" The student should ask, "Is there a specific biomarker?"

KEY SCIENCE TOPICS: This lesson is derived from lab practical skill 7 from A Level biology: Electrophoresis as a separation process. This practical focuses on using complex procedures and apparatus, evaluation skills are an intended learning outcome.

OVERALL TIME ESTIMATE: Two 45 minute class periods

LEARNING STYLES: Kinesthetic

VOCABULARY:
Proteomics: large scale study of proteins, their structure and function.

Gel Electrophoresis: the lab process of separating molecules of interest from a tissue sample. The molecules are DNA, RNA or proteins.

Hypothesis: a proposal intended to explain certain facts or observations. Testable or verifiable statement.

LESSON SUMMARY: This lab will be an inquiry based lab using a gel electrophoresis to determine if tissue samples from different autopsies test positive or negative for the IL-1ra protein. If this protein is not present, than it presumes the patient died from a lack of this suppressor molecule and that once the baby suffered from a leaky gut, the mechanisms to reduce the swelling was not present. If the tissue tests positive for the gene, (kD 20 band) then one must assume there are other mechanisms that may create a leaky gut along with IL-1ra. The students will tabulate and evaluate their data. They will post their findings for the other benches to use for lesson 5 and 6.

STUDENT LEARNING OUTCOMES for AICE BIOLOGY
The student will be able to...
1. Set up and run a gel electrophoresis for the purposes of finding a specified protein.
2. Determine if their specimen tests positive or negative for the protein of interest.
3. Write an evaluation based upon their findings and draw a conclusion as to whether their work supports the hypothesis or not.

STANDARDS

PRIOR KNOWLEDGE: Students must be familiar with the equipment to run a gel electrophoresis.

MATERIALS:
- ESSENTIAL: Bio-Rad Biotechnology Explorer kit: Comparative Proteomics Kit 1: Protein Profiler Module catalog # 166-2700EDU. This kit will contain the reagents necessary for this lab for 8 benches, however there are required accessories:
  o 1-2 PowerPac Basic power supply to four gel boxes at once (#164-5050EDU)
  o 8 2-20 ul adjustable volume micropipette
  o 1 gallon Distilled or deionized water
  o 5-6 different types Fish samples, 1 gram per bench
  o 1 pair of Scissors or knife to cut fish samples
  o 1 Water bath or heat block at 100C
  o 4 Mini-PROTEAN 3 electrophoresis module (gel box) runs one or two gels (#165-3302EDU)
BACKGROUND INFORMATION: Proteomics is the study of proteins, their structure and function. This lab will be determining if a tissue sample (in this case, fish tissue samples will be run, but they will be referred to as patient samples) has a molecule of interest. Gel Electrophoresis is a technique used to separate molecules by charge. The gels determine if a tissue sample tests positive or negative for a molecule of interest; DNA, RNA or protein. However, each of those molecules has a different protocol and process. This lab focuses on determining if there is a specific protein in a sample or not, therefore this lab will focus on running a protein gel. The protein of interest being sought for this lab is the IL-1ra. The hypothesis being tested is: If a tissue sample tests negative for the protein IL-1ra, then the lack of this protein is a causal factor in the mortality rate for NEC babies.

For this lab, the students will be given tissue samples from different patients. All of these samples are from autopsies of babies that died from NEC. Different benches will test the same patients. They will match their results along with a control sample that will test positive for IL-1ra. The students will use the ExD protocol in the appendix to write up their lab results. They will tabulate and evaluate their own work. They will then post their findings on a large poster along with the other benches. They will need this information for assignment 5 and 6.

ADVANCE PREPARATION

1. Follow the kit instructions for complete set up of lab.
2. Obtain 5 different fish samples, suggested samples are: shark, sturgeon, catfish, trout and salmon. 1 gram of each fish sample per bench is needed.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

Day 1

Teacher Preparation: While the students are preparing their samples, or just before class, the gel boxes need to be set up for loading. The instructions are:

1. Set up Mini-PROTEAN 3 gel box and add 1xTGS electrophoresis buffer to the chamber.
2. Prepare a Ready Gel cassette by cutting along the black line on the bottom of the cassette with a razor blade and pulling off the plastic strip, as indicated on gel cassette.
3. Remove the comb from the Ready Gel cassette.
4. Place ready Gel cassette into the electrode assembly with the short plate facing inward. Place a buffer dam or another Ready Gel cassette on the opposite side of the electrode assembly, with notch on buffer dam facing inward.
5. Slide gel cassette, buffer dam and electrode assembly into the clamping frame.
6. Press down the electrode assembly while closing the two cam levers of the clamping frame.
7. Lower the inner chamber into the mini tank.
8. Completely fill the inner chamber with 1x TGS electrophoresis buffer, making sure the buffer covers the short plant (~150ml).
9. Fill mini tank approximately 200 ml of 1xTGS electrophoresis buffer.
10. Plate sample loading guide on top of the electrode assembly.

Teacher Instructions:

1. Electrophorese for 30 minutes at 200 V in 1x TGS electrophoresis buffer.
2. After electrophoresis, remove gel from cassette and place two gels in one staining tray. Rinse gels in tap water. If time allows rinse gels 3 times for 5 minutes.
3. Pour off water and add 50 ml of Bio-Safe Coomassie blue stain. Stain gels for at least 1 hour with gentle shaking for best results.
4. Discard stain and destain gels in a large volume of water overnight, changing the water at least once. Blue stained bands will be visible on a clear gel after destaining.

Student Instruction:
1. (10 minutes) Follow kit instructions for lab steps 1-6.
2. While the samples are in the water bath, the students will prepare to run their gels. The gel boxes need to be set up before by the instructor.
3. Obtain the samples after they have been heated to 95°C for 5 minutes.
4. (20 minutes) Load your gel using the following guidelines.

<table>
<thead>
<tr>
<th>Lane</th>
<th>Volume</th>
<th>Sample</th>
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<tbody>
<tr>
<td>1 &amp; 2</td>
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<tr>
<td>3</td>
<td>5uL</td>
<td>Precision Plus Protein Kaleidoscope prestained standards</td>
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<tr>
<td>4</td>
<td>10uL</td>
<td>Sample 1</td>
</tr>
<tr>
<td>5</td>
<td>10uL</td>
<td>Sample 2</td>
</tr>
<tr>
<td>6</td>
<td>10uL</td>
<td>Sample 3</td>
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<td>7</td>
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<td>Sample 4</td>
</tr>
<tr>
<td>8</td>
<td>10uL</td>
<td>Sample 5</td>
</tr>
<tr>
<td>9 &amp; 10</td>
<td>Empty</td>
<td>Empty</td>
</tr>
</tbody>
</table>

5. This will take 40 minutes. The teacher will finish by adding the stains and allowing them to stain overnight.

Day 2
1. (10 minutes) Students will obtain their gels and examine the bands.
2. The IL-1ra band is 20kD. This is the band that they will be comparing their samples to.
3. The students will make a judgment on whether the different tissue samples test positive or negative for the IL-1ra protein.
4. They will present their findings in ExD format in their lab notebook. The protocol is found in the appendix and a sample is included with assessments suggestions. Allow rest of class period.

ASSESSMENT SUGGESTIONS:

- Students are to turn in their lab using the ExD format only. This format is included in the appendix for this curriculum. Therefore the students will be assessed on the completion of this lab. It should resemble the following:
  I. Title: Why did my baby die?
  II. Observation: This will be 2-3 sentences along the lines of a relationship between the lack of IL-1ra and the rate of mortality for NEC babies.
  III. Hypothesis: If the tissues test negative for the IL-1ra protein molecule, then this can be determined as a contributing factor in the mortality of the NEC babies.
  IV. Presence or absence of IL-1ra DV: mortality rate due to NEC
  IV. Procedures: The students will list the apparatus and a brief review of the procedures of the experiment.
     Control group: Precision Plus Protein Kaleidoscope prestained standards, IL-1ra gene band is kD 20
     Experimental group: the patient samples
  V. Results: This should be represented in a table showing the patients and the results
  VI. Conclusions: This is an evaluation of their procedures including the possibility of any errors and how any errors may affect the results. The students will conclude with whether their results support their hypothesis.
  VII. Extension: Students will write a brief paragraph on possible extensions, need for replication or other explorations to validate their hypothesis.

RESOURCES/REFERENCES:
• Biotechnology Explorer™ Comparative Proteomics Kit 1: Protein Profiler Module. Bio-Rad Catalog number 166-2700 EDU

LESSON THREE - Is my baby going to be alright?

KEY QUESTION(S): When parents are faced with the unexpected premature delivery of a neonate, the baby is often underdeveloped and at a low birth weight. Sometimes these babies are confined to the NICU until the risks of infections and independent living are established. One of the most frequently asked questions becomes, "Is my baby going to be alright? Is he/she at risk?" The student should ask, "Can I find the biomarker in a non invasive tissue sample?"

KEY SCIENCE CONCEPTS: The dot blot is a technique for detecting, analyzing and identifying proteins. This lab will show the concentration of proteins in a crude preparation (such as a culture supernatant) and be estimated semi-qualitatively by using a specific antibody to identify it. Therefore, this lab is used to test tissue sample of babies to determine if they are missing or have a low level of IL-1ra.

OVERALL TIME ESTIMATE: one 45 minute class period

LEARNING STYLES: Kinesthetic

VOCABULARY:
Dot blot: a diagnostic test to determine if a molecule of interest is present in a tissue sample. The molecule must have a known antibody.

Supernatant: the usually clear liquid lying above a solid residue after crystallization, precipitation, centrifugation or other processes.

Nitrocellulose membranes: a lab grade paper with a sticky side to immobilize nucleic acids or proteins

Buccal swabs: by swabbing with a cotton swab on the inside of the cheek to obtain a cell sample

Antibody-antigen complex: a known coupling of an antigen with an antibody. These are highly specific and usually related in terms of the immune response. An antigen would be a foreign or non self molecule and the leukocytes will attach a matching/fitting molecule to disable the antigen from doing any harm.

Primary antibody: molecules that recognize and bind to a specific protein, they are used to target biological molecules of interest.

Secondary antibody: molecules that will bind to a specific primary antibody, these will have a florescent tag associated with them. When a UV light is shown on the secondary antibody with a florescent tag, then they will glow.

LESSON SUMMARY: The students are going to a Dot Blot lab to determine if the neonates in NICU are at risk for developing NEC. The dot blot is a test that will determine if a patient is carrying a specific protein by first purifying the protein, matching it will a specific primary antibody and then matching the primary antigen with a secondary antigen with a UV conjugated antibody that will glow when a UV light is shown on it. When the students have their data, they will tabulate and evaluate it as part of the ExD. Then they will post their data for the other benches to use for lesson 5 and 6.

STUDENT LEARNING OUTCOMES for AICE BIOLOGY
The student will be able to...
1. Set up and run a dot blot test for the purposes of finding a specified protein
2. Determine if their specimen tests positive or negative for the protein of interest.
3. Write an evaluation based upon their data and draw a conclusion as to whether their results support the hypothesis or not.
STANDARDS:

PRIOR KNOWLEDGE: Students need to be able to use pipettes. They need to have an understanding of antibody-antigen complex.

MATERIALS:

- **ESSENTIAL** (per 2 person bench):
  - Nitrocellulose membrane
  - Incubating Tray
  - Serum from buccal swab
  - Blocking Solution (Block)
  - Primary Antibody (1° Ab)
  - UV-conjugated Secondary Antibody (2°Ab)
  - TBS-T Solution (Wash)
  - UV Light

BACKGROUND INFORMATION: The dot blot test is a relatively quick assay to determine if a specific protein is in a supernatant. The mixture containing the molecule is applied directly on a nitrocellulose membrane as a dot, and then is plotted through circular templates directly onto a membrane or paper substrate. This does not offer information on the size of the molecule, as a gel electrophoresis would, but it will confirm the presence or absence of a molecule targeted. The molecule of interest is the IL-1ra obtained from the buccal swab of a neonate. The molecules must already have an **antibody-antigen complex** that is known. When the molecule is placed on this membrane, a **primary antibody** wash is poured all over the membrane. The antibodies will attach themselves to the antigen (molecule of choice). A **secondary antibody** wash is then washed over the primary antibody. The secondary antibody will have a UV sensitive molecule attached to when the secondary antibody attaches to the primary antibody that has attached to the antigen (molecule of interest) and when placed under a UV light, it will glow. That means that specimen has the IL-1ra.

With this lab, when the students test the different tissue samples from buccal swab. If the sample from the baby glows, that would indicate a positive result and means that they are at a low risk for developing NEC. If a test comes back negative, that baby could be at high risk for developing NEC.

ADVANCED PREPARATION

- This lab is a simulation. There are no tissues or proteins being tested. Instead, a nitrocellulose membrane has been prepared using a UV pen to predetermine a set of data. However, the students should not be provided with this initial information until after the lab is finished. Although they will know that the samples are not real neonate buccal samples, they can be led to believe they are protein samples from other sources.
- Provide the following for each lab bench:
  - 1 Nitrocellulose membranes to blot samples (Bio-Rad, Sample cat # 162-0146) gridded for 12 samples. The grids should be labeled with small circles numbered 1-8, some of them need to be randomly marked with an invisible UV pen (pens must be waterproof)
  - To simulate the results the following marking is recommended (number 1 will always be positive control and number 2 will always be negative control):
    - Bench 1: # 1,3,5,7
    - Bench 2: # 1, 3,7
    - Bench 3: # 1,3,4,5,7
    - Bench 4: # 1,5,7,8
    - Bench 5: # 1,3,5,7,8
    - Bench 6: # 1,3,5,7
    - Bench 7: # 1,3,5,7,8
- Bench 8: # 1,5,7
- Bench 9: # 1,3,4,5,6,7,8
- Bench 10: # 1,3,7
- Bench 11: # 1,5,7,8
- Bench 12: # 1,3,5,7

- Serum Samples in microtubes
  - 1 positive control: water with small amount of red food coloring
  - 1 negative control: water with small amount of red food coloring
  - 6 samples from buccal swabs: water with small amount of food coloring and a small ‘pellet’ in the bottom of the sample to represent tissue. These should be in different microtubes labeled with numbers and initials to indicate patients.

- Reagents
  - Block: water with a few drops of milk
  - Wash: water with a very small amount of detergent
  - Primary Ab: water
  - Secondary Ab: water with a small amount of blue food coloring
  - Pipettes for dropping serum samples, label the pipettes 1-8 to avoid confusion and to reinforce that new pipettes must be used for each patient sample

**PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES**

1. Check your lab station for the following:
   a. Nitrocellulose membrane grid with washing tray
   b. 8 micro tubes with one positive control, negative control and 6 different patients
   c. Reagents: block, wash, primary Ab, secondary Ab
   d. Pipettes labeled 1-8

2. (2 minutes) Spot 2 drops of serum of each sample onto the nitrocellulose grid. Make sure that you keep track of what sample you are marking in what circle.

3. Place the membrane in the tray and allow drying for 2 minutes.

4. Cover the membrane with block, so that it is barley immersed. Gently agitate. Allow 1 minute to block.

5. Dump the block and pour the primary antibody until the membrane is immersed. Gently agitate. Allow to incubate for 2 minutes.

6. Dump the primary antibody and rinse the membrane with the wash for 30 seconds submersing the membrane and gently agitating for 30 seconds. Do this 3 times.

7. Place the membrane back in the tray and pour secondary antibody so that the membrane is submerged. Gently agitate for 1 minute.

8. Dump the secondary antibody and wash 3x for 30 seconds each time.

9. Allow the membrane to dry for 5 minutes before checking your samples. If the IL-1ra is present, the sample will glow under a UV light, if the sample does not glow, then the patient is lacking the IL-1ra and is high risk for developing NEC.

10. Procedures, results and conclusions should be recorded in your lab notebook.

**ASSESSMENT SUGGESTIONS:**
Students are to turn in their lab using the ExD format only. This format is included in the appendix. Therefore the students will be assessed on the completion of this lab. It should resemble the following:

I. Title: Is my baby going to be alright?
II. Observation: This will be 2-3 sentences along the lines of a relationship between the lack of IL-1ra and the risk assessment for developing NEC.
III. Hypothesis: If the sample tests negative for the IL-1ra protein molecule, then this can be determined as a possibility of a higher risk of developing NEC.
   IV: presence or absence of IL-1ra
   DV: risk assessment of developing NEC
IV. Procedures: The students will list the apparatus and a brief review of the procedures of the experiment.
   Control group: positive and negative control sample provided
   Experimental group: the patient samples
V. Results: This should be represented in a table showing the patients, number of trials run and the positive or negative results.
VI. Conclusions: This will not exceed one paragraph, but the student will write an evaluation of procedures, if they think there were any errors and what effect the errors would have had on the results.
VII. Extension lab: This is a proposal of what the student feels would come next.

EXTENSIONS:
ACTIVITIES: Students should list hypothetical extensions in their ExD.

RESOURCES/REFERENCES:

- Houda Darwiche. Center for Precollegiate Education Training, Dot Blot Simulation
LESSON FOUR: Please help my baby!

KEY QUESTION(S): When parents are faced with the unexpected diagnosis of NEC, they are upset, confused and full of guilt. Sometimes these babies are confined to the NICU under sterile conditions and some babies will need surgery. The risks of surgery are also high in that it may result in extended developmental problems and delays. When a parent faces the fact their baby is ill, the most frequent pleas a doctor will then hear is, "PLEASE HELP SAVE MY BABY!" so the question is, "How do you calculate the correct dosage for different birth weights?"

KEY SCIENCE CONCEPTS: This lesson will focus on the concept of using a serial dilution to determine the correct proportion of probiotics and medicine to help the babies that have been diagnosed with NEC. These babies already have been determined to have a low or non existent level of IL-1ra. This lab will focus on the correct proportion and delivery of probiotics and medicine to help reduce the swelling in the leaky gut and promote the healing of NEC and promote a healthier microbiota in the babies gut.

OVERALL TIME ESTIMATE: one 45 minute class period

LEARNING STYLES: Kinesthetic

VOCABULARY:
Serial dilution:

probiotic:

dosing:

anti-inflammatory:

LESSON SUMMARY: The students are going to do a serial dilution lab to determine the correct ratio of probiotics to medicine for the most effective healing of the gut. The probiotics will introduce commensal microbiota and the medicine will help in reducing the leaky gut by reducing the swelling. Too much or too little of one or the other and it could prove fatal. Therapeutic medicines must often be dosed by weight and within a certain range for most effective healing.

STUDENT LEARNING OUTCOMES for AICE BIOLOGY
The student will be able to...
1. Set up and run a serial dilution to determine different concentrations that are effective within a range.
2. Calculate the concentrations needed for three different birth weights.
3. Write an evaluation based upon their lab and draw a conclusion as to whether their work supports the hypothesis or not.

STANDARDS:

MATERIALS
• ESSENTIAL:
  o buffer pH 10, 10 ml per bench
  o Buffer pH 3, 10 ml per bench
  o Phenol red indicator 1ml per bench
  o 2 ml tubes (10 per bench)
  o Tube rack
  o White tile
  o 3 1ml syringes
**BACKGROUND INFORMATION:** A **serial dilution** is a stepwise dilution of a substance in solution. The dilution factor is always kept at a constant progression of a logarithmic fashion. A ten fold serial dilution starts at 1.0 M, 0.1 M, 0.01 M, 0.001 M and 0.0001 M. Other dilutions are in ratios; such as 20:80, 30:70 and so on. Both of these dilutions create an obvious difference in concentration of a solution. This concentration will be a deciding factor for proper proportion of the treatment, how much probiotics suspended in how much medicine. **Dosing** applies to the presentation or feeding an organism in small enough amounts at different intervals to determine at what concentration a treatment will become effective without being too much or too little. **Probiotics** are live microorganisms that are thought to be beneficial to a host organism. They are positively contributing to the gut microflora for proper digestion, absorption and are currently being investigated to alleviate chronic intestinal inflammatory diseases. **Anti inflammatory** medication is used to reduce swelling in a tissue, to help normalize the organ.

**ADVANCE PREPARATION:**

- Each bench will need:
  - buffer pH 10, 10 ml per bench: LABEL SOLUTION A
  - Buffer pH 3, 10 ml per bench: LABEL SOLUTION B
  - Phenol red indicator 1ml per bench: LABEL P
  - 2 ml tubes (10 per bench)
  - Tube rack
  - White tile
  - 3 1ml syringes

**PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:**

**Student instructions:**

1. Label 7 of the 2 ml tubes 1 through 7

2. Label your syringes A and B. Do not confuse them.

3. Create a serial dilution using solution A and B. A is the medicine and B is the probiotic. This will be accomplished by using the following formula:
   - Tube 1: 1 ml A
   - Tube 2: 0.8 ml A and 0.2 ml B
   - Tube 3: 0.6 ml A and 0.4 ml B
   - Tube 4: 0.5 ml A and 0.5 ml B
   - Tube 5: 0.4 ml A and 0.6 ml B
   - Tube 6: 0.2 ml A and 0.8 ml B
   - Tube 7: 1 ml B

4. Add one drop of P to each tube.

5. Note the color changes.
   - Pink indicates not enough probiotics and too much medicine
   - Yellow indicates too much medicine and not enough probiotics
   - Orange indicates the proper range of the ratio needed.

6. Using 5 fresh tubes; do a further dilution. Be sure to indicate the amount of solution A and B in each tube.

7. Add one drop of P to each tube.

8. Note color change.
9. Decide on the range of ratios you could use to treat the sick babies.

10. Calculate the proper dosage for a baby weighing 500g, 750g, 1250 g and 1500 g.
   a. Use the formula: 1 ml dose per 1000 grams baby weight
   b. Be sure to include how much of solution A and solution B to use for each weight.

**ASSESSMENT SUGGESTIONS:**

- Students are to turn in their lab using the ExD format only. This format is included in the appendix. Therefore the students will be assessed on the completion of this lab. It should resemble the following:
  I. Title: Please save my baby!
  II. Observation: This will be 2-3 sentences along the lines of a relationship proper dosing of probiotics and anti inflammatory drugs to the babies affected with NEC.
     Hypothesis: The correct dose of probiotics and medicine will help reduce the inflammation of the leaky gut and help promote healing of NEC.
     IV: correct dose of probiotics and medicine
     DV: decrease in NEC signs and symptoms
  III. Procedures: The students will list the apparatus and a brief review of the procedures of the experiment. Control group: none Experimental group: the patient samples
  VI. Results: This should be represented in a table showing the results of the serial dilutions
  V. Conclusions: This will not exceed one paragraph, but the student will write an evaluation of procedures, if they think there were any errors and what effect the errors would have had on the results.
  VII. Extension lab: This is a proposal of what the student feels would come next.

**EXTENSIONS:**

**ACTIVITIES:** Students should list hypothetical extensions in their ExD.

**RESOURCES/REFERENCES:**
LESSON FIVE: Results of Your Research

KEY QUESTION(S): Did your research support your hypothesis? Did you get the same results as other benches?

SCIENCE CONCEPTS: This lesson covers Assessment Objectives Part B of the AS Level AICE Biology 2013 curriculum. Candidates should be able to:
1. Locate, select, organize and present information from variety of sources (the other benches)
2. Translate information from one form to another (creating tables or graphs)
3. Manipulate numerical and other data.
4. Use information to identify patterns, report trends and draw conclusions.
5. Give reasoned explanations for phenomena, patterns and relationships.
6. Make predictions and hypothesis.
7. Apply knowledge, including principles, to new situations.
8. Demonstrate an awareness of the limitation of biological theories and models.

OVERALL TIME ESTIMATE: Introduced at the beginning of a 45 minute class period, extended into homework if necessary.

LEARNING STYLES: Prose, writing

VOCABULARY: Results

LESSON SUMMARY: After the students have performed their 3 labs, they will then be expected to create a Results paper. This paper will address the learning objectives from the AICE syllabus as the student draws the information from their labs and compares it to the other lab groups, or benches. They will focus on just one of the labs, create an extended amount of data, then present it in a tabulated or graph form with a short paragraph written in an objective voice describing their findings.

STUDENT LEARNING OBJECTIVES (AS STATED IN THE TEACHING A2 BIOLOGY PRACTICAL SKILLS)
There are 7 Practical Skills that contribute to the overall understanding of scientific methodology. By the end of the course, it is expected that candidates will be able to carry out or follow an investigation. In a scientific investigation these would be applied in the following sequence:
1. Planning the experiment
2. Setting up/manipulating apparatus
3. Making measurements and observations
4. Recording and presenting observations and data
5. Analyzing the data and drawing conclusions
6. Evaluating procedures
7. Evaluating conclusions

STANDARDS

MATERIALS:

- ESSENTIAL:
  - Data from all three experiments
  - Data from the other benches
BACKGROUND INFORMATION: After the students have completed the three labs, they will have an incredible amount of data and may not know what to do with it all. Therefore, they will be writing a results paper. This paper will give them the opportunity to aggregate the data of the other lab groups, or benches in all the AICE bio classes. A results paper is a collection of quantitative data, there is no opinion, nor conclusion. This paper will include either a table or a graph that depict the results of what the students found and then compared and contrasted to the other benches. The paper will consist of an introduction, the data presentation, and then an analysis and evaluation. It will not be longer than two pages.

ADVANCE PREPARATION:

1. There is a student handout that is included with this unit. Each bench should have a copy of how to write the results paper. Students should also be able to access the directions and lab data electronically through the school website or other site.
2. All labs must be completed and lab stations cleaned.
3. All of the lab data from each bench should be on poster paper in the classroom.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. (5 minutes) Hand out the student paper for lesson 5, the results paper. This paper also includes lesson 6, the conclusion, since the students will follow the writing of one right after the other.
2. (10 minutes) Present the directions on the screen and go over the instructions. Answer any questions.
3. Allow the students the rest of the class period to work on their papers.
4. Teacher should check on groups and students independently to make sure students are on task and answer any problems.

ASSESSMENT SUGGESTIONS:

- Only 2 of the objectives will be measured and assessed with this paper:
  - Recording and presenting observations and data
  - Analyzing the data and drawing conclusions
- The student will receive marks by having tabulated or graphed their data so that it is in a presentable form. These must include: title, identification of each axis, logical numbering or column headings, a key for reference, date. Each of those will be assessed at 2 points a piece for a total of 10 points. An analysis, as defined by the AICE curriculum is to be able to express information and ideas in a clear and accurate style; the ability to identify key points in a study and see their relationship to other studies and theories; and the ability to make valid generalizations. For a good analytical result, they will receive another 10 points.

RESOURCES/REFERENCES:

- Shuttleworth, Martyn. How to Write a Research Paper. Published by Oskar Blakstad and Experimental-Resources.com. 2010
LESSON SIX: How are you going to tell them?

KEY QUESTION(S): If you were a doctor or principle investigator in a lab, how would you present your findings? Present your conclusions

KEY SCIENCE CONCEPTS: This lesson covers Assessment Objectives Part B of the AS Level AICE Biology 2013 curriculum. Candidates should be able to:
1. Locate, select, organize and present information from variety of sources (the other benches)
2. Translate information from one form to another (creating tables or graphs)
3. Manipulate numerical and other data.
4. Use information to identify patterns, report trends and draw conclusions.
5. Give reasoned explanations for phenomena, patterns and relationships.
6. Make predictions and hypothesis.
7. Apply knowledge, including principles, to new situations.
8. Demonstrate an awareness of the limitation of biological theories and models.

OVERALL TIME ESTIMATE: Introduced at the beginning of a 45 minute class period, extended into homework if necessary.

LEARNING STYLES: Prose, writing

VOCABULARY:
Conclusions: after an analysis of the data, one draws a conclusion by summarizing the key points and presenting them in a clear coherent style. A good conclusion will also include an evaluation of the data, taking into account possible errors and the effects they would have on data.

LESSON SUMMARY: After the students have presented their results, they will now choose either the role of a medical doctor or a principal researcher PhD from a lab and they will present their conclusions.
M.D.: The student will write a conclusion based on their findings as if they are speaking with parents of a pre-term neonate that has been diagnosed with NEC. They will write one paragraph on the background, one paragraph on their lab findings and three paragraphs on what they recommend the parent’s course of action should be based on their analysis and evaluation.
PhD: The student will write a conclusion based on their findings as if they are speaking to a cohort group of other researchers. One paragraph on background, one paragraph on their results and three paragraphs on whether they think their research should be continued to be funded and expanded based on their analysis and evaluation.

STUDENT LEARNING OBJECTIVES (AS STATED IN THE TEACHING A2 BIOLOGY PRACTICAL SKILLS)
There are 7 Practical Skills (see appendix) that contribute to the overall understanding of scientific methodology. When the investigation is complete, students should be able to create an analysis and evaluation based on their results. This should be written in a clear and concise style and include any perceptions of possible errors and the effects they could have on the data.

STANDARDS:

MATERIALS:

- ESSENTIAL:
  - Data from all three experiments
  - Data from the other benches
  - Their Results paper
BACKGROUND INFORMATION: After the students have written their results paper, they will write a conclusion paper. A conclusion starts with a broad approach and ties everything together. At the end of a conclusion, the student should write a recommendation for follow up research or a course of action. This will be the concluding remarks to the entire unit.

ADVANCE PREPARATION:

1. There is a student handout that is included with this unit it covers lesson 5 and 6. Each bench should have a copy of how to write the conclusion paper. Students should also be able to access the directions and lab data electronically through the school website or other site.
2. All labs must be completed and lab stations cleaned
3. All of the lab data from each bench should be on poster paper in the classroom.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. (5 minutes) Hand out the student paper for lesson 6, the conclusion paper.
2. (10 minutes) Present the directions on the screen and go over the instructions. Answer any questions.
3. Allow the students the rest of the class period to work on their papers.
4. Teacher should check on groups and students independently to make sure students are on task and answer any problems.

ASSESSMENT SUGGESTIONS:

- Only 2 of the objectives will be measured and assessed with this paper:
  o Evaluating procedures
  o Evaluating conclusions
- As defined by the AICE curriculum, evaluation skills are defined in terms of the ability to point out methodological errors and consider their effect on the data; the ability to consider the quality of the data; the ability to consider the ethics of the study and the ability to consider the scientific value of the outcome of the study.
- The student’s papers will cover assessment #6 in paragraph 2; this is where the present their findings and will by that time realize that not every student got the same results. So now the student is faced with making judgment calls on whether their experiment carries validity or not.
- Paragraph #3 will cover an evaluation of conclusions. This will be covered when the student makes a recommendation. They will be assessed on whether they could support their findings and make another judgment call to either the parents or peers.

REFERENCES:

- Shuttleworth, Martyn. How to Write a Research Paper. Published by Oskar Blakstad and www.Experimental-Resources.com. 2010
APPENDIX

Experimental Design Template

All lab work is to be turned in using the following template. This template is central dogma to the way the scientific method is applied to discover new things, validate findings, eliminate variables and create a greater scientific knowledge base. Do not deviate from this protocol. Do not fudge or make up results. No results or unexpected results are not wrong results. You must use Roman numerals and the bold font words.

I. **Title:** must be absolutely definitive
II. **Observation:** something that you have seen or worked with and are looking for an answer.
III. **Hypothesis:** If it is easier for you, create one using the If/then format. If will be your independent variable, then will be the dependent variable.
   a. Label your **IV:** this is what you are manipulating
   b. Label your **DV:** this is what is being is measured
IV. **Procedure:** this can be limited in words, do not get too bogged down in detail
   a. List **control group** and **experimental group**
   b. How many trials are being run
V. **Results:** either graphed or tabulated with a key
VI. **Conclusion:** not more than a paragraph
VII. **Extension lab:** since science is never finished, where could these conclusions lead you next?

*Example*

I. **Title:** Where did all the fish in the lake go?
II. **Observations:** It seems over the last year, that the number of fish is declining in a neighborhood lake. At the same time the amount of algae seems to be growing at a very rapid rate. The lake is surrounded by nice homes with perfect fertilized lawns. We are going to sample different lakes with differing concentration of algae to see if there is a correlation between the concentration of algae and the number of fish. Algae blooms are known to decrease the concentration of dissolved oxygen in the water and this could be the cause of the reduction in the amount of fish.
III. **Hypothesis:** The greater concentration of algae in a lake results in a decrease in the concentration of dissolved oxygen. If there are high concentrations of algae in a lake, then the concentration of dissolved oxygen will be lower than normal.
   a. **IV:** concentration of algae in a specified area of a lake
   b. **DV:** concentration of dissolved oxygen at that site
IV. **Procedure:** We will be taking water samples from different lakes in the area. We will use a healthy lake that is situated back in a nature preserve that is known for its good fishing as a control. We will then sample other lakes that have algae blooms as our
experimental groups. In the experimental conditions, we will sample water under the blooms and in areas away from the blooms.

a. Control group: healthy lake water samples
b. Experimental group: water samples from lakes with algae blooms and known to not have many fish.
   i. Experimental groups will have samples from under the blooms and away from the blooms.
   ii. 5 samples from each algae bloom area will be run and a mean score tabulated.

V. Results:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lake control</th>
<th>Lake #1</th>
<th>Lake #2</th>
<th>Lake #3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algae</td>
<td>Na</td>
<td>56% DO</td>
<td>65% DO</td>
<td>45% DO</td>
</tr>
<tr>
<td>Non-algae</td>
<td>97% DO</td>
<td>78% DO</td>
<td>82% DO</td>
<td>75% DO</td>
</tr>
</tbody>
</table>

VI. Conclusions: The areas of the lakes with the greater algae blooms have an overall lower percentage of DO. These areas seem to have less fish than the control. The higher the dissolved oxygen in a lake seems to be a direct indicator of the number of fish reported in the lake. Fish rely on a high concentration of dissolved oxygen in the water.

VII. Extension lab: Could the levels of nitrogen be affecting the growth of algae? The next labs will measure the levels of nitrogen in the affected areas to see if there is a higher level of nitrogen that may be affecting the growth of the algae.
Teaching A Level Practical Skills

What are the practical skills required by this course?

This course addresses seven practical skills that contribute to the overall understanding of scientific methodology. In a scientific investigation these would be applied in the following sequence.

1. Planning the experiment
2. Setting up / manipulating apparatus
3. Making measurements and observations
4. Recording and presenting observations and data
5. Analysing data and drawing conclusions
6. Evaluating procedures
7. Evaluating conclusions

The syllabus shows how these seven skills are assessed and the structure is common to all three sciences. The emphasis of the AS syllabus is on developing an understanding and practice of scientific procedures, the collection of data, analysis and drawing conclusions. It also starts to develop critical evaluation of procedures by suggesting improvements to experimental procedures. In general students find the performance of practical procedures and the collection of data more accessible than analysis, whilst evaluation is least readily accessed. To enable access to these more demanding skills, students need to understand why an experimental procedure is carried out in a particular way so that they can recognise sources of error or limitations which could affect the reliability of their results. Students will not be able to evaluate until they can critically review a practical procedure.

The A2 syllabus builds upon the skills developed in AS and its emphasis is on the higher level skills of planning, analysis and evaluating. In order to plan effectively, students need to be able to evaluate procedures and critically assess results. This is best achieved by the performance of practical exercises starting in AS with relatively straightforward and familiar contexts and developed in A2 by the use of more complex procedures and less familiar contexts. Data analysis again develops from AS into more complex treatments so that students need to be given opportunities to gather suitable data and perform the appropriate manipulations. The evaluation of conclusions and assessing procedures are very high order skills. Students who have not had sufficient opportunity to plan and trial their own investigations will find these skills difficult. Students are not expected to be able to plan perfectly, but to recognise weaknesses and make reasonable suggestions for improvement. The best learning tool to develop these skills is to devise a plan, carry out the investigation and then assess how well the planned procedure worked. The syllabus gives detailed guidance on the expected skills and learning outcomes.

In summary, as the syllabus clearly shows, skills 2-6 listed above will be assessed at AS level in papers 31 and 32. Skills 1 and 7 will only be assessed at A level in paper 5, which will also take skills 5 and 6 to a higher level.
The above list shows the seven skills in the order in which they would be used in an extended investigation. It is not suggested, nor would it be wise, to teach these skills in this order. Students who are new to practical work will initially lack the basic manipulative skills, and the confidence to use them. It would seem sensible, therefore, to start practical training with skill 2, initially with very simple tasks and paying attention to the establishment of safe working practices. Once a measure of confidence in their manual dexterity has been established, AS students can move on to exercises that require skills 3 and 4 to be included. Extensive experience in carrying out practical procedures allows students to gain awareness of appropriate quantities and become more organised in time management and the recording of data as it is collected. It is likely that skill 6, Evaluating Procedures, will be the most difficult to learn at AS level. Critical self-analysis does not come easily to many people. ‘My experiment worked well’ is a frequent and inappropriate response. If students are to master this skill, they need to develop an appreciation of reliability and accuracy inherent in the equipment and procedure they are using. Only then will they be able to identify anomalous results, or results which fall outside of the ‘range of uncertainty’ intrinsic in Teaching A2 Biology Practical Skills the choice of apparatus used and so are considered to be inaccurate. Exercises with less reliable/accurate outcomes can be used to provide more scope for the evaluation of procedural, technique or apparatus errors.

Planning is arguably the most demanding of the seven skills. For it to be effective, students need to be very well grounded in skills 2-6, so that they can anticipate the different stages involved in the task, and can provide the level of detail required. It is for this reason that planning skills are not assessed at AS level but form part of the A2 assessment in Paper 5. Unless students use apparatus they do not develop an understanding of how it works and the sort of measurements that can be made using particular sorts of apparatus. Candidates cannot be taught to plan experiments effectively unless, on a number of occasions, they are required:

- to plan an experiment;
- to perform the experiment according to their plan;
- to evaluate what they have done.

The evaluation of conclusions, skill 7, is done by comparison of the outcome of an exercise with the predicted outcome, and so is also an A2 skill. It should be taught and practised as part of the planning exercises.

Summary of each of the 7 skills
Full details of the requirements for each of these skills may be found on pages 34 to 41 of the syllabus. What follows below is a brief summary of the skills involved.

1. Planning
   a. Defining the problem: Students should be able to use information provided about the aims of the investigation, or experiment, to identify the key variables. They should use their knowledge and understanding of the topic under consideration to make a quantitative, testable, prediction of the likely outcome of the experiment.
   b. Methods: The proposed experimental procedure should be workable. It should, given that the apparatus is assembled appropriately, allow data to be collected without undue difficulty. There should be a description, including diagrams, of how the experiment
should be performed and how the key variables are to be controlled. Equipment, of a level of precision appropriate for the measurements to be made, and quantities to be used should be specified. The use of control experiments should be considered.

c. Risk assessment: Candidates should be able to carry out a simple risk assessment of their plan, identifying areas of risk and suggesting suitable safety precautions to be taken.

d. Planning for analysis, conclusions and evaluation: Students should be able to describe the main steps by which their results would be analysed in order that valid conclusions might be drawn. This may well include the generation of a results table and the proposal of graphical methods to analyse data. Also, they should propose a scheme for the interpretation and evaluation of the results themselves, and of the experimental procedure employed in obtaining those results. There should be an indication of how the outcomes of the experiment would be compared with the original hypothesis.

2. Setting up / manipulating apparatus

a. It is important that students are allowed sufficient time and opportunity to develop their manipulative skills to the point where they are confident in their approach to experimental science. They must be able to follow instructions, whether given verbally, in writing or diagrammatically, and so be able to set up and use the apparatus for experiments correctly.

3. Making measurements and observations

a. Measuring/observing: Whilst successfully manipulating the experimental apparatus, it is crucial that students are able to make measurements with accuracy and/or to make observations with clarity and discrimination. Accurate readings of meters or burettes and precise descriptions of colour changes and precipitates will make it much easier for students to draw valid conclusions, as well as scoring more highly in the test.

b. Deciding on what measurements/observations to make: Time management is important, and so students should be able to make simple decisions on the number and the range of tests, measurements and observations that can be made in the time available. For example, if the results of the first two titrations are in good agreement, there is no need to carry out a third. Students need to be able to make informed decisions regarding the appropriate distribution of measurements within the selected range, which may not always be uniform, and the timing of measurements made within the experimental cycle. They should also be able to identify when repeated measurements or observations are appropriate. The strategies required for identifying and dealing with results which appear anomalous should be practised.

4. Recording and presenting observations and data: An essential, but frequently undervalued, aspect of any experimental procedure is the communicating of the results of the procedure to others in a manner that is clear, complete and unambiguous. It is vital that students are well practised in this area.

a. The contents of the results table: The layout and contents of a results table, whether it is for recording numerical data or observations, should be decided before the experiment is performed. ‘Making it up as you go along’ often results in tables that are difficult to follow and don’t make the best use of space. Space should be allocated within the table for any manipulation of the data that will be required.
b. The column headings in a results table: The heading of each column must be clear and unambiguous. In columns which are to contain numerical data, the heading must include both the quantity being measured and the units in which the measurement is made. The manner in which this information is given should conform to ‘accepted practice’.

c. The level of precision of recorded data: It is important that all data in a given column is recorded to the same level of precision, and that this level of precision is appropriate for the measuring instrument being used.

d. Display of calculations and reasoning: Where calculations are done as part of the analysis, all steps of the calculations must be displayed so that thought processes involved in reaching the conclusion are clear to a reader. Similarly, where conclusions are drawn from observational data, the key steps in reaching the conclusions should be reported and should be clear, sequential and easy to follow.

e. Significant figures: Students should be aware that the number of significant figures to which the answer is expressed shows the precision of a measured quantity. Therefore, great care should be taken with regard to the number of significant figures quoted in a calculated value. The general rule is to use the same number of significant figures as (or at most one more than) that of the least precisely measured quantity.

f. Data layout: Students should be able to make simple decisions concerning how best to present the data they have obtained, whether this is in the form of tabulated data or as a graph. When plotting graphs they should be able to follow best practice guidelines for choosing suitable axis scales, plotting points and drawing curves or lines of best fit. In drawing tables they should be able to construct a table to give adequate space for recording data or observations.

5. Analysing data and drawing conclusions: This skill requires students to apply their understanding of underlying theory to an experimental situation. It is a higher-level skill and so makes a greater demand on a student’s basic understanding of the biology involved. Even when that understanding is present, however, many students still struggle. The presentation of a clear, lucid, watertight argument does not come naturally to most people and so much practice in this area is recommended.

a. Interpretation of data or observations: Once data has been presented in the best form for analysis of the results of the experiment, the student should be able to describe and summarise any patterns or trends shown and the key points of a set of observations. Further values such as the gradient of a graph may be calculated or an unknown value found, for example from the intercept of a graph.

b. Errors: Students should be used to looking at an experiment, assessing the relative importance of errors and where appropriate, expressing these numerically. Students should be aware of two kinds of error.

i. The ‘error’ that is intrinsic in the use of a particular piece of equipment. Although we refer to this as an equipment error, we really mean that there is a ‘range of uncertainty’ associated with measurements made with that piece of equipment. This uncertainty will be present no matter how skilled the operator might be.

ii. Experimental error, which is a direct consequence of the level of competence of the operator or of the effectiveness of the experimental procedure.
c. Conclusions: Students should learn to use evidence to support a given hypothesis, to draw conclusions from the interpretation of observations, data or calculated values and to make scientific explanations of their data, observations and conclusions. Whatever conclusions are drawn, they must be based firmly on the evidence obtained from the experiment. At the highest level, students should be able to make further predictions and ask appropriate questions based on their conclusions.

6. Evaluating procedures: Arguably, this is one of the most important, and probably one of the most difficult skills for a student to develop. In order for the evaluation to be effective, students must have a clear understanding of the aims and objectives of the exercise, otherwise they will not be able to judge the effectiveness of the procedures used. They must be able to evaluate whether the errors in the data obtained exceed those expected due to the equipment used. If this is the case, they then need to identify those parts of the procedure which have generated these excess errors, and suggest realistic changes to the procedure which will result in a more accurate outcome. Students should also be able to suggest modifications to a procedure to answer a new question.

a. The evaluation procedure may include:
   i. the identification of anomalous values, deducing possible causes of these anomalies and suggesting appropriate means of avoiding them,
   ii. an assessment of the adequacy of the range of data obtained
   iii. an assessment of the effectiveness of the measures taken to control variables
   iv. taking an informed judgement on the confidence with which conclusions may be drawn.

7. Evaluating conclusions: This is also a higher-level skill, which will demand of the student a thorough understanding of the basic theory that underpins the science involved. The conclusions drawn from a set of data may be judged on the basis of the strength or weakness of any support for or against the original hypothesis. Students should be able to use the detailed scientific knowledge and understanding they have gained in theory classes in order to make judgements about the reliability of the investigation and the validity of the conclusions they have drawn. Without practice in this area, students are likely to struggle. In order to increase the confidence in drawing conclusions, it is recommended that practical exercises, set within familiar contexts, be used to allow students the opportunity to draw conclusions, make evaluations of procedure and assess the validity of their conclusions. In the examination, students may be required to demonstrate their scientific knowledge and understanding by using it to justify their conclusions.
Lesson 5: Results Paper
Lesson 6: Conclusion Paper

According to the AICE syllabus, candidates should be able to handle information and solve problems, using oral, written, symbolic graphical and numerical forms of presentation. Therefore, you will keep in mind the following learning objectives and create a results paper and a separate conclusion paper. When you have finished the three labs, you should be able to:

1. Locate, select, organize and present information from variety of sources (the other benches)
2. Translate information from one form to another (creating tables or graphs)
3. Manipulate numerical and other data.
4. Use information to identify patterns, report trends and draw conclusions.
5. Give reasoned explanations for phenomena, patterns and relationships.
6. Make predictions and hypothesis.
7. Apply knowledge, including principles, to new situations.
8. Demonstrate an awareness of the limitation of biological theories and models.

Results are simply a presentation of your findings. It is a straight forward commentary on exactly what you have found in your experiments. There is no opinion, no conclusions.

For this assignment, you will present quantitative data in the form of a table or graph and statistical data from either lab 1, 2 or 3. You will also include all of the data from the other benches (lab groups). You will first compare and contrast your data with the other benches, then make an overall presentation of data. You must include and results that do not support your hypothesis. These are called negative results.

Tables: These must be clear and succinct. It should be easy enough and conclusive enough to sum up the entire work. It should include enough as it needs and no more than necessary.

Decide on your format. You will need to figure out how many trials were run and what the results were for each of them. You may consider putting the benches on one axis and either a positive or negative result for the other axis. Be sure to sum up the table in one or two sentences. Provide a key if necessary.

Graphs: helpful to reinforce main points. Make sure to include an error bar if there is a range. Be sure to include a key.

Write a short paragraph that explains your results. This should be done in an objectified voice.

Conclusion: For a conclusion paper, you will either choose the voice of an M.D. or a PhD. A medical doctor needs to relay the results of the research to the parents of a pre-term neonate.
The parents are faced with a baby with positive results for NEC. As a PhD, you will write a paper to other researchers asking them to replicate your study for confirmation, or to manipulate a variable for a more comprehensive approach.

The paper should only be one page, three paragraphs long. Start with a broad look regarding the state of research for NEC, discuss your findings, and narrow it down to what you feel the next step should be.

You may hand in these assignments together, but on separate sheets of paper.