Chronic Inflammation:
The Dark Side of the Inflammatory Process

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OVERVIEW

This unit is written for a high school introductory biology course for 20 students. The unit is designed to be implemented at the end of the course, so students already have learned about cells, tissues, organs, genetics, blood and immune system basics. Any (or all) of the introductory and review pieces can be easily left out, and thus, it can be modified for higher-level students who are already familiar with some of the topics.

This is a Project Based Learning (PBL) type of activity which is used to teach the importance of preventing a chronic inflammatory state by avoiding *excess* nutrients and practicing good dental hygiene. Students will read a **mock** letter addressed to the teacher from the Community Education Coordinator of a fictitious local community health center/hospital. The letter will explain that the center would like to add the subject of chronic inflammation to a series of community educational programs. The letter goes on to state that because the topic deals with the very complicated immune system, doctors have reported that they struggle when trying to explain this to their patients. They are seeing increasing cases of nutrition-related obesity and poor oral hygiene/health which connected to a chronic inflammatory state. They have found that the general public has a poor understanding of chronic inflammation (CI) and the roles that obesity and dental infections, such as periodontitis and gingivitis, may play. The coordinator requests that the students prepare posters to display at an event. The hope is that the students can put this complex and detailed information into layman's terms and organize it in a way that the typical community member (who has a high school education that includes biology) can easily understand. All posters will be inspected for accuracy and be approved by a panel of physicians before the event. Students should be able to explain the information on their poster.

After students read the letter and discuss this as a class, they will review how they are going to learn the necessary information needed to prepare the poster. Students will brainstorm what they already know about the inflammatory process (the expectation is that they have already learned this as part of a previously covered immune system unit) and what they already know about chronic inflammation (they may know nothing about this yet; this will be addressed in these lesson plans). They will then pose questions about what they need to learn. This will be documented in a KQA chart (similar to the commonly used KWL chart). It may be necessary for the class to review the basics of the Immune System before learning about chronic inflammation. The teacher can use the PowerPoint lesson that is included in Day 2 to briefly review the inflammatory process and teach students about chronic inflammation.

Next, students will need to prepare to learn how to perform an ELISA to determine IL-6 levels, an inflammatory marker. They will first learn about what an ELISA is and how it is used. Then, they will need to learn and practice some lab techniques specific to ELISA, such as micropipetting and working with microvolumes of solutions. Then, they will actually perform the ELISA lab and semi-quantitatively determine the IL-6 levels in the simulated serum sample of 2 hypothetical patients. One will have an elevated level, while the other will have no detectable IL-6. This lab activity will take 3 days to complete, and there is a fair amount of "downtime." Students will consider a possible relationship between elevated levels of IL-6 and how obesity and dental infections can be a source of chronic inflammation. Finally, they will prepare the group poster according to the rubric.

Concerning the poster presentation at the "mock" hospital's community educational health meeting, the teacher will arrange for adults (and possibly other students) in the school to represent typical members of the community. The students who have completed this lesson plan will then be present next to their posters to explain and answer questions.

Student Outcomes and Learning Objectives

Students will:

1) review prior knowledge.
2) learn about chronic inflammation, some of its causes, and how it can contribute to the onset of a few diseases.
3) practice common lab techniques, as well as learn and practice new lab techniques such as serial dilution and micropipetting.
4) practice working with microquantities of reagents.
5) learn how to make serial dilutions.
6) learn about how an ELISA could be used to determine a marker of CI. They will perform an ELISA assay (a detailed, multi-step extended laboratory protocol) to semi-quantitatively determine the presence of human IL-6 antigen in simulated samples of human serum of 2 fictitious patients. IL-6 can be found at an elevated level in some cases of chronic inflammation.
7) work collaboratively to prepare a poster explaining chronic inflammation and possible connection to both obesity and poor dental health
8) present the poster and be able to answer questions about its content to a group of peers and non-peers (community members)

This is relevant to students because obesity and poor dental health, and thus chronic inflammation, are becoming more and more of a human health problem

Educational Standards:

Next Generation Science Standards (NGSS)

- Develop and use a model based on evidence to illustrate the relationships between systems or between components of a system (HS-LS-1-2)
- Use a model based on evidence to illustrate the relationships between systems or between components of a system (HS-LS1-4), (HS-LS1-5), (HS-LS1-7)
- Plan and conduct an investigation individually and collaboratively to produce data to serve as the basis for evidence, and in the design: decide on types, how much, and accuracy of data needed to produce reliable measurements and consider limitations on the precision of the data (e.g., number of trials, cost, risk, time), and refine the design accordingly. (HS-LS1-3)
- Systems of specialized cells within organisms help them perform the essential functions of life. (HS-LS1-1)
- Multicellular organisms have a hierarchical structural organization, in which any one system is made up of numerous parts and is itself a component of the next level. (HS-LS1-2)
- Feedback mechanisms maintain a living system's internal conditions within certain limits and mediate behaviors, allowing it to remain alive and functional even as external conditions change within some range. Feedback mechanisms can encourage (through positive feedback) or discourage (negative feedback) what is going on inside the living system. (HS-LS1-3)
- Empirical evidence is required to differentiate between cause and correlation and make claims about specific causes and effects. (HS-LS4-2),(HS-LS4-4),(HS-LS4-5)

Vermont Standards: Grade Expectations

Enduring Knowledge: The human body is unique in its heredity, body systems and development and can be affected by the environment.

S9-12:42

Students demonstrate their understanding of the Patterns of Human Health/Disease by…
Identifying a variety of nonspecific means of protection for the human body and explaining how these maintain human health (i.e., prevent disease).

AND

Explain how the general process of the human **immune system** responds to foreign substances and organisms (e.g., phagocyte action and antibody production and maintenance).

AND

Showing through models/diagrams/graphic organizers how specific biological abnormalities alter the normal functioning of human systems

AND

(EXTENSION)
Explaining the effect of unique viral diseases on the cells of the human **immune system** (e.g., retroviruses).

Science Concepts:

a. The Human Body protects itself against infectious diseases (caused by microorganisms, viruses, animal parasites) through physical protection and physiological (**immune**) responses.
b. The Immune System is designed to protect against microscopic organisms (bacteria, fungi) and foreign substances that enter from outside the body and against some cancer cells that arise within.
c. Some allergic responses are caused by the body's immune responses to usually harmless environmental substances.
d. Humans have a variety of mechanisms—sensory, motor, emotional, social and technological—that can reduce and modify health hazards (e.g. blinking, fight or flight, coping mechanisms, medicine).
e. The severity of human disease depends upon many factors, such as resistance to disease and the virulence of the infecting organism.
f. Biological abnormalities, such as injuries or chemical imbalance, cause or increase susceptibility to disease (e.g. hormonal imbalance, epilepsy, depression).

Time Requirements

As written, this unit will take 12 days of 90 min classes. More time may be needed if the teacher chooses to do a basic immune system review prior to Day 1.

Target audience and expected prior knowledge:

This unit is written for a high school introductory biology course for 20 students. The unit is designed to be implemented at the end of the course, so students already have learned about cells, tissues, organs, genetics, blood and immune system basics.

Students should be familiar with general laboratory materials, equipment and have basic lab skills, such as measuring and transferring reagents. They should also be familiar with general lab etiquette, protocol and safety.

Any (or all) of the introductory and review pieces can be easily left out and thus it can be modified for higher-level students who are already familiar with some of the topics.

Teacher Section

Materials, Reagents, and Equipment

Many of the following can be purchased from a number of biological supply companies. I have noted the vendors that I used when developing the lab. Exception: The ELISA Ready Set Go Kit –Human IL-6 must be ordered from eBioscience

Material	Vendor/Item no.	Cost	Quantity
ELISA Ready Set Go Kit –Human IL-6 Contains: coating buffer diluent enzyme detection antibody coating antibody substrate	eBioscience 88-7066-22 2x96 tests (comes with 2 plates, enough reagents for 112 wells) www.eBioscience.com	$209.00 (if you call customer service, they might give a discount) 888.999.1371	1 kit
Bottles, jars, or any suitable container that can be capped or covered. a) To make up solutions b) For aliquots of some solutions I like to use plastic conical test tubes with caps.	The conical test tubes are available in several sizes from any Flinn Scientific or Carolina Biological **Test Tubes with Screw Caps, Plastic, 30/pkg** **Flinn Cat# -Ap-7116**	14.95/pkg 30	a)100 mL -1 25 mL -1 30 mL -3 b)15 mL
Microcentrifuge tubes- a) to prepare standards serial dilutions b) aliquot some solutions for student workstations	Microcentrifuge Tube, Natural, 500/pkg Flinn FB0002 -	$19.05 500/pkg	1.5 mL
STOP solution TMB STOP solution (alternative: 2N H_2SO_4 or 1M H_3PO_4) Safety caution : strong acidic solution	KPL (for TMB stop) www.kpl.com Product code 50-85-05	$63.90	400 mL (this is much more than needed, but the smallest amount sold)
96-well plate	eBioscience (Nunc Maxisorp flat-bottom, Cat. No. 44-2404)	$2.75	The kit comes with 2 plates –order enough extra so that each student has their own
ELISA Plate sealers	eBioscience DY992	69.00/pack of 100	Pack of 100
Micropipets	www. edvotek.com 1-800-EDVOTEK a) Cat # 591-1 b) Cat # 592-1	a) $179.00 b) $179.00 at least 1 of each for each group of 4 students	a) 20-250 µL b) 100-1000 µL
Micropipette tips	www. edvotek.com 1-800-EDVOTEK a) Cat # 636-B b) Cat # 637	a) 1-200 µL b) 200-1000 µL	a) Bag of 1000 tips b) Bag of 1000 tips
Refrigerator dedicated to biology/chemical samples (It is not safe to store chemicals and biological specimens in refrigerators where edibles may be kept)			
ELISA Wash Buffer	eBioscience Cat# 00-0400	$119/10 packets	1 unit of 10 packets
Washing bottles (to dispense ELISA wash buffer solution)	Flinn AB-8109	$4.65 each	500 mL (5)

Lesson Plan Overview

Before you begin this unit review the immune system if necessary with the website noted or use your own resources/ideas

Day #1
- A. Entry Event (read mock letter)
- B. Define the problem (discuss mock letter)
- C. Anticipation Guide
- D. Discuss "Road map" –How will we learn what we need to know?

Day #2
- L1 (if this review is used and done during class time, it may add another day to the unit)
- L2 Review the inflammatory process and learn about chronic inflammation

Day #3
- L3a ELISA videos, demonstrations, intro to equipment

Day #4
- L3b Micropipetting and "flick and blot" practice

Day #5
- L3c ELISA lab Day 1
  - a) Review procedure and materials
  - b) Complete ELISA protocol Steps 1 & 2 a, b, c

Day #6 L3c ELISA lab Day 2
- a) Steps 1 and 2

Day #7 L3c ELISA lab Day 3
- a) Steps 1 a-f

Day #8 L3c ELISA lab Day 4
- a) Steps 1, 2, 3, 4

Day #9
- Lab Analysis and Discussion, Review, Begin to prepare poster

Day 10, 11
- Prepare poster

Day 12
- Culminating event: The Mock Community Health Event
  Poster Session for Chronic Inflammation

Note: Day 5 should be a Friday, Monday, or Tuesday.
Day 6, 7 8 must be done on consecutive days.

Entry event:

Dear (teacher's name):

I am the Coordinator of Community Education at Mountain Valley Regional Medical Center. We are currently working on the schedule for a series of upcoming community education sessions. One of the topics that I would like to present is Chronic Inflammation (CI). As this topic deals with the very complicated immune system, doctors have reported that they struggle when trying to explain this to their patients. They are seeing increasing cases of nutrition-related obesity and poor oral hygiene/health and are concerned about the connection of these to a chronic inflammatory state. Doctors have found that the general public has a poor understanding of CI and the roles that obesity and dental infections, such as periodontitis and gingivitis, may play.

I have heard about some of the service projects that your students have done in the past. I am asking if perhaps your students would be willing to prepare posters to display at our event. The hope is that they can put this complex and detailed information into layman's terms and organize it in a way that the typical community member (who has a high school education that includes biology) can easily understand. Please understand that all posters will be inspected for accuracy and must be approved by a panel of physicians before display. Students should be able to explain what is on their poster.

Please discuss this with your students and call me at 802-331-MVMC when you have made a decision. We are looking forward to working with you.

Sincerely,

Margaret Davis
Community Education Coordinator

Define the Problem

Discuss this idea with the students.
Cases of nutrition-related obesity, gingivitis and periodontitis are on the rise in the local community. These conditions can cause CI, but this may be preventable through good nutrition and oral hygiene.

During the discussion, students may come up with the following question. If not, ask leading questions to guide them to it.
Can we, as a class of biology students, help to increase public awareness of this issue by preparing posters to be used at the local hospital community education event?

KQA Anticipation Chart

What do we already know about chronic inflammation and what do we need to learn?

K What do we already **know** about a) the inflammatory process? b) chronic inflammation?	**Q** What **questions** do we have about a) the inflammatory process? b) chronic inflammation?	**A** What are the **answers** to these Questions? (to be filled in at the end of the project)

Road Map

How will we learn the answers to our questions and how will we get to the final product? :

1) Review the immune system –if necessary

The following web site is a good review of immune system basics
http://www.biology.arizona.edu/immunology/tutorials/immunology/main.html

2) Review the Inflammatory Process and learn about Chronic Inflammation (CI)

PPT/lecture /discussion

3) Perform LAB activities

Expected prior knowledge: Students should be familiar with general laboratory materials, equipment and have basic lab skills, such as measuring and transferring reagents. They should also be familiar with general lab etiquette, protocol and safety.

a) Pre-lab: Introduction to sandwich ELISA --- Do together as a class --

- Preview the following 4 videos to learn the ELISA techniques.
- Then, watch any or all of the following 4 videos together as a class. (I have chosen these
- 4, but there are many good videos / tutorials / animations available on the web.)
- Have materials available to show and discuss with students as they are shown in the video (well plates, micropipetters, microcentrifuge tubes).
- Stop at various points and demonstrate and/or review with class to be sure they understand.
- Students should answer the questions during the review.

You may also choose to assign these for homework the night before.

Explain that they are going to do this procedure, but not use the platereader for quantitation. (It is unlikely that a typical high school would have this piece of equipment.)

We will be doing a semi-quantitative visual colorimetric determination of IL-6 concentration instead.

1. We will be doing the Sandwich Elisa.
   https://www.youtube.com/watch?v=2-sVA_b-ivQ This video is good because he also refers to 2 other uses of this technique: for a pregnancy test and HIV test
2. https://www.youtube.com/watch?v=nNjlBCnpGZ4
3. Virtual sandwich ELISA lab activity (**Amrita University)**
   http://amrita.vlab.co.in/index.php?sub=3&brch=69&sim=699&cnt=1332 Note: You may have to log in as guest, but you only need to give starred information. Students should see the Sandwich ELISA animation.
4. http://www.hhmi.org/biointeractive/immunology-virtual-lab

b) Preparation for ELISA lab:

Learn skills and techniques necessary to perform ELISA

Training labs:

Micropipetting lab and practice working with microquantities

http://www.biotech.iastate.edu/publications/lab_protocols/PipettorHampton.pdf

--this is a good activity to help students hone micropipetting skills

Students can print out the pages from this link themselves and then complete the lab with your assistance.

c) ELISA Lab

Semi-quantitatively measure IL-6 in the fictitious serum of a person with elevated IL-6 and a disease condition that may have been a consequence of the chronic inflammatory state [Type 2 diabetes (from impaired insulin signaling), some cancers, Alzheimer's, stroke].

Each group will receive a previously prepared fictitious patient sample and patient profile with medical information and a table with reference values for IL-6. The task will be to prepare serially diluted quantitative IL-6 standards and determine the patient's IL-6 value range.

Teacher Advance Preparations

Several solutions will need to be prepared before they will be used.

Materials needed for advance prep:

Material	**Quantity**
Bottles, jars, or any suitable (should not be glass or polystyrene) container that can be capped or covered. I like to use plastic conical test tubes with caps.	100 mL -1 25 mL -1 30 mL -3
Micropipets	a)20-250 µL b) 100-1000 µL
Micropipette tips	10

If prepared more than two hours in advance, solutions should be stored in the refrigerator at 4°C (unless otherwise noted).

Preparation *	**Prepare on Day # __ of ELISA lab**	**Students will use on Day # __ of ELISA lab**	**How to prepare**	**Estimated prep time**
ELISA Wash Buffer	1	2,3,4	Label a 1L bottle or other suitable container "ELISA Wash Buffer" (container should not be glass or polystyrene) Dissolve powder and dilute in distilled water according to package directions. Repeat 3 times to prepare a total of 3L Label 5-500 mL wash bottles "ELISA Wash Buffer" Aliquot 500 mL to each of 5 wash bottles (each lab group of 4 students can share 1)	15 min
1x Diluent	1	2,3,4	Label a bottle "1x Diluent" Add 20 mL of 5x diluent to 80 mL of distilled water. Rock multiple times to mix thoroughly. Aliquot 10 mL into each of 10-15 mL labeled conical test tubes or vials	5 min
1x Coating buffer	1	Students will not use this. This will be used for you to dilute the Capture antibody	Label a small bottle or other suitable vessel "1x Coating Buffer" (vessel should not be glass or polystyrene) Add 2.5 mL of 10x coating buffer to 22.5 mL of distilled water. Rock multiple times to mix thoroughly (or follow supplier directions if using a powder)	15 min

Capture antibody	1	1	Label a small bottle or other suitable vessel "Capture Antibody" (vessel should not be glass or polystyrene) Add 120µL of stock Capture antibody to 30 mL of 1x coating buffer. Rock multiple times to mix thoroughly Aliquot 6 mL into each of 5 labeled conical test tubes or vials (4 students can share one)	15 min
Detection antibody	4	4	Label a small bottle or other suitable vessel "Detection Antibody" (vessel should not be glass or polystyrene) Add 120µL of stock Detection antibody to 30 mL of 1x Diluent Rock multiple times to mix thoroughly Aliquot 6 mL into each of 5 labeled conical test tubes vials (4 students can share one)	15min
Enzyme	4	4	Label a small bottle or other suitable vessel "Enzyme" Add 120µL of stock Enzyme to 30 mL of 1x Diluent Rock multiple times to mix thoroughly. Aliquot 6 mL into each of 5 small labeled test tubes or vials (4 students can share one)	15 min
Substrate	Working concentration (as supplied in kit)	4	Aliquot 4 mL into each of 5-15mL conical test tubes. Label "Substrate"	5 min
STOP solution	Working concentration (as purchased)	4	Aliquot 4 mL into each of 4 15mL conical test tubes. Label "STOP"	5 min
"Top" IL-6 standard (200pg/mL)	2	2	Label a 15-mL conical test tube or other suitable container "IL-6 top standard 200pg/mL" (container should not be glass or polystyrene) 1) Reconstitution: Add 1mL of distilled water to vial of lyophilized standard. Allow to sit for 15min with periodic gentle agitation (concentration of standard is now 15ng/mL) Note: you will use 100µL of this to prepare the top standard in step 2 below, as well as 50µL to prepare Patient #1 sample 2)Dilution: Add 100µL of reconstituted standard to7400 µL of 1X Diluent (concentration of standard is now 200 pg/mL) Aliquot 1 mL to each of to 5 microcentrifuge tubes. Label "200 pg/mL"	30 min

Patient #1 sample (will have 100 pg/mL IL-6)	2	4	Label a 15-mL conical test tube or other suitable container “Patient #1 Sample” (container should not be glass or polystyrene) Add 50μL of reconstituted standard to7400 μL of 1X Diluent (this makes Patient #1 sample with a concentration of 100 pg/mL) Aliquot 1 mL to each of to 5 microcentrifuge tubes. Label “Patient #1”	
Patient #2 sample (will have no detectable IL-6)	2	4	Aliquot 1 mL of ELISA 1X Diluent to each of 5 microcentrifuge tubes. Label “Patient #2”	

***For a class of 20 students, each student will have their own plate with 8 wells used (5 standards, two patient samples and one negative control). Students should work in groups of 4.**

Standards serve as positive controls

If refrigerated, all solutions should be brought to room temperature before using.

ELISA Lab: Day 1

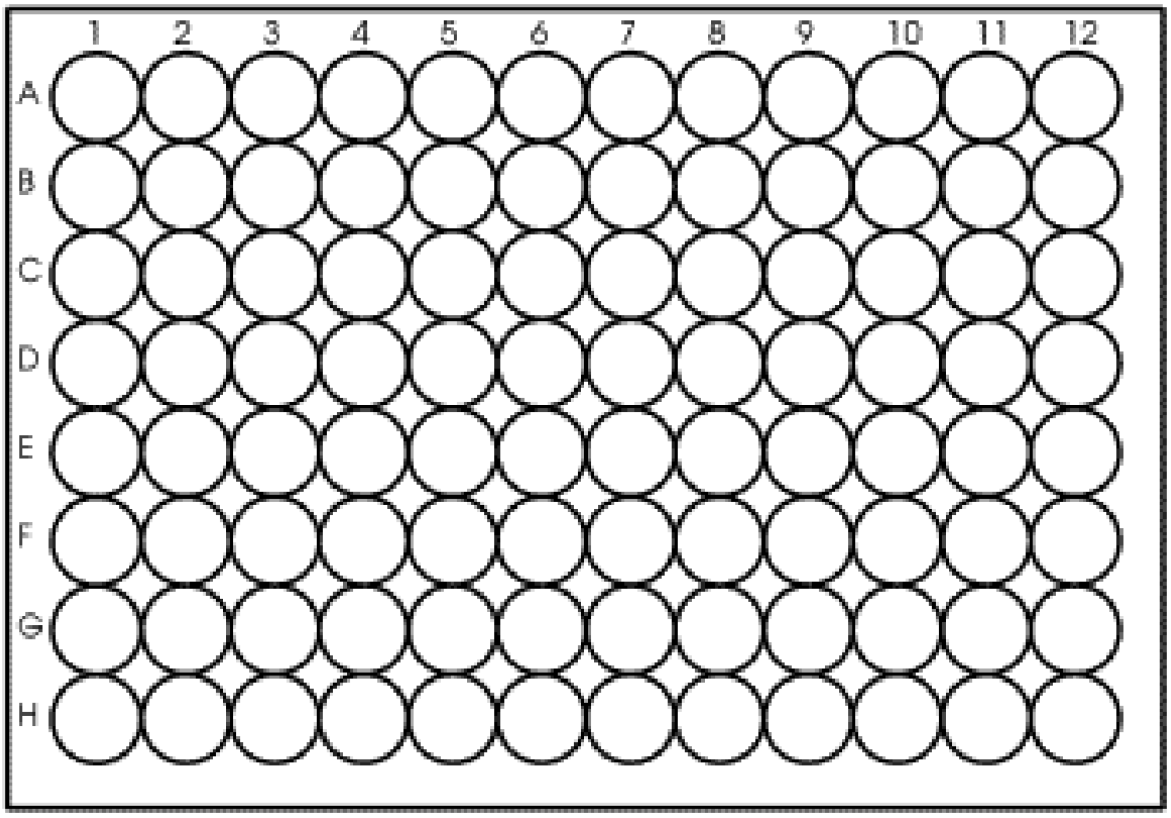
STUDENT WORKSTATION CHECKLIST

Each work station should have the following materials for a group of 4 students.

Item	Contents/volume	Number/amount
ELISA plates		4 (1 per student)
ELISA plate sealers		4 (1 per student)
micropipetters	Capable of delivering 100, 200 and 250 µL	1-4 (students can share, but ideally each student would have their own)
micropipetter tips		A supply of at least 50 tips
sink or wash bucket		1
Capture antibody		1 microcentrifuge tube aliquot
Paper toweling		1 roll
PERSONAL SAFETY PROTECTION		4 of each: Goggles, aprons, gloves

1. Label the ELISA plate template below as follows:

Well	Contents	Write this on template
A-1	IL-6 standard 200 pg/mL	200
B-1	IL-6 standard 100 pg/mL	100
C-1	IL-6 standard 50 pg/mL	50
D-1	IL-6 standard 25 pg/mL	25
E-1	IL-6 standard 12.5 pg/mL	12.5
F-1	1x ELISA diluent (Blank)	0 - Blank
G-1		Patient #1
H-1		Patient #2



2. Coat wells A-H of the ELISA plate with the Capture Antibody:

a. Add 100µL of the Capture Antibody to each well.
b. Seal the plate with the plate sealer.
c. Incubate in refrigerator at 4C overnight.

ELISA Lab: Day 2

STUDENT WORKSTATION CHECKLIST

Each work station should have the following materials for a group of 4 students.

Item	Contents/volume	Number/amount
micropipetters	**Capable of delivering 100, 200 and 250 µL**	**1-4 (students can share, but ideally each student would have their own)**
1x Diluent		**1 conical test tube, 10 mL Diluent**
micropipetter tips		**A supply of at least 50 tips**
sink or wash bucket		**1**
ELISA wash buffer	**500 mL**	**1 wash bottle**
Top IL-6 standard		**1 micro centrifuge tube aliquot**
Mini centrifuge tubes (to prepare serial dilutions of standard)		**20 (5 per student)**
Paper toweling		**1 roll**
PERSONAL SAFETY PROTECTION		**4 of each: Goggles, aprons, gloves**

1. Wash and block:

a) Remove the liquid from the wells by "flick method." (demonstrated in the video/animation)

SECURELY hold the ELISA plate in the palm of your hand flat then pretend that you have a baseball (or fly swatter) and quickly snap your arm propelling your hand towards the sink.

Blotting is best done with some force. Holding the plate in your hand, bring it down to "hit" the stack of napkins, but not so hard as to break the plate
DEMONSTRATE this for students. It would be helpful for them to practice with plates and water.

Do not let the wells dry out. Add the liquid for the next step promptly.

b) Wash 3 times with Wash Buffer (demonstrated in the video/animation)

- Fill each well using a squirt bottle.
- Allow wells to soak in Wash Buffer for approximately 1 min during each wash step.
- Flick out the liquid.
- Blot plate on a thick layer of absorbent paper to remove any residual buffer

c) Block wells by adding 200µL 1X ELISA Diluent to each well.

d) Seal the plate with the plate sealer.

e) Incubate in the refrigerator at 4°C overnight.

2. In the meantime, prepare the standards

Prepare 4 serial dilutions of the IL-6 "top standard" (200 pg/mL) in Eppendorf tubes as follows:

- Label Eppendorf tubes A(200), B(100), C(50), D(25), E(12.5).
- Add 250 µL of ELISA Diluent to Eppendorf tubes B-E.
- Add 500 µL of the "top standard" to tube A.
- Remove 250 µL from tube A and transfer to tube B and mix by pipeting up and down. Tube B now contains the 100 pg/mL standard.
- Remove 250 µL from tube B and transfer to tube C and mix by pipeting up and down. This tube now contains the 50 pg/mL standard.
- Remove 250 µL from tube C and transfer to tube D and mix by pipeting up and down.This tube now contains the 25 pg/mL standard.
- Remove 250 µL from tube D and transfer to tube E and mix by pipeting up and down. This tube now contains the 12.5 pg/mL standard.
- Remove 250 µL from tube E and dispose of it so that all tubes contain 250 µL total**.**
- Incubate in the refrigerator at 4°C overnight.

ELISA Lab: Day 3

Item	Contents/volume	Number/amount4
ELISA plates		4 (1 per student)
micropipetters	Capable of delivering 100, 200 and 250 µL	1-4 (students can share, but ideally each student would have their own)
micropipetter tips		A supply of at least 50 tips
sink or wash bucket		1
Standards that were prepared on Day 2		Each student has their own set they prepared
Patient sample #1		1 microcentrifuge tube, aliquot
Patient sample #2		1 microcentrifuge tube, aliquot
1x Diluent		1 conical test tube, 10 mL Diluent
Paper toweling		1 roll
Wash buffer		1 - 500 mL in squeeze wash bottle
PERSONAL SAFETY PROTECTION		4 of each: Goggles, aprons, gloves

1. Add STANDARDS, Patient SAMPLES, and BLANKS to well.

a) Remove the blocking liquid from the wells by flicking. Promptly add the liquid for the next step.
b) Add 100 µL of each of the 5 standards prepared yesterday to the appropriate wells according to the Well Template.
c) Add 100 µL of the Elisa Diluent to serve as blanks to the appropriate wells according to your Well Template.
d) Add 100µL of each of your patient samples to the appropriate wells according to the Well Template.
e) Seal the plate with the plate sealer.
f) Incubate at 4C overnight.

ELISA Lab: Day 4

Item	Contents/volume	Number/amount
micropipetters	Capable of delivering 100, 200 and 250 µL	1-4 (students can share, but ideally each student would have their own)
micropipetter tips		A supply of at least 50 tips
sink or wash bucket		1
Capture antibody		1 microcentrifuge tube aliquot
Enzyme		1 microcentrifuge tube aliquot
Substrate		1 microcentrifuge tube aliquot
STOP solution		1 microcentrifuge tube aliquot
Paper toweling		1 roll
Wash buffer		1 - 500 mL in squeeze wash bottle
PERSONAL SAFETY PROTECTION		4 of each: Goggles, aprons, gloves

1. Add the DETECTION ANTIBODY

a) "Flick and wash" as on Day 2 in steps 1a and b. Repeat for a total of 3 washes.
b) Add 100 µL/well of the Detection Antibody to each well.
c) Seal the plate with the plate sealer.
d) Incubate at room temperature 1 hr

2. Add the ENZYME

a) "Flick and wash" as on Day 2 in steps 1a and b. Repeat for a total of 3 washes.
b) Add 100 µL/well of the Enzyme.
c) Seal the plate with the plate sealer.
d) Incubate at room temp 30 min

3. Add the SUBSTRATE and the STOP solution

a) "Flick and wash" as on Day 2 in steps 1a and b. Repeat for a total of 5 washes.
b) Add 100 µL of Substrate (1X TMB) to each well.
c) Watch carefully now for the first hint of blue color in well C1.
d) Quickly, but steadily add 100 µL of STOP Solution to each well, beginning with A1 and following the same order that you have been doing.

Note: It is important to add the STOP solution in the same order as the substrate and at the same steady pace.

4. Determine IL-6 concentration

a) Determine the approximate range of IL-6 in your Patient samples by visually comparing them to the standards.

b) Assign a range based on the color intensity. For example, if the color intensity of Patient Sample 1 falls in between that of Std #2 and Std #3, you would determine the IL-6 value for Patient 1 to be between 50 – 100 pg/µL.

	Known IL-6 level, pg/mL	**Approximate IL-6 range (visually compared to standards)**
Std #1	200	----------
Std #2	100	----------
Std #3	50	----------
Std #4	25	----------
Std #5	12.5	----------
Blank	0	----------
Patient #1	----------	
Patient #2	----------	

In this laboratory, considering limitations of this test, we will consider any detectable level of IL-6 to be an *elevated level*. The normal range of IL-6, with this test, is below the detectable levels, or in this case, 0 pg/mL. Elevated levels of IL-6 can be due to a number of reasons, one of which may be the presence of a chronic inflammatory state. (In this case, the patient's care provider may choose to investigate further and order more tests. It is important to realize that a single test does not always provide the complete story!)

You are given the following information about each patient:
Patient #1 is 28 years old. Patient has recently reported constant "excruciating" pain from area in mouth "throughout lower jaw." Area seems tender to the touch. Patient reports recurring "toothaches" in that area during the past "year or so –usually flares up about every six weeks." At those times, patient self-medicates with over the counter products –"usually Tylenol or Advil, which provide relief, but this time, those medications have not been effective in relieving the pain at all."
Visual examination reveals swelling and redness of the lower right jaw and general poor dental hygiene. Patient admits that friends and family often remark that he has "bad breath." Patient reports last dental visit 10 years ago.

Patient #2 is 18 years old and is 190 lbs. Normal weight for patient of this age, height and build is 140 lbs. Patient complains of being "tired all the time and hungry a lot."
When questioned about diet, patient admits to poor nutritive choices

- eats very few fruits, vegetables and whole grains
- chooses mostly high fat, highly processed foods
- drinks no water
- usually soda and energy drinks
- often eats at fast food restaurants and usually orders the "super-size" option.

Conclusion Questions:

1. Based on your IL-6 test results and the patient information, do you think the care provider for Patient #1 would have reason to consider additional testing? Explain your answer. Please use complete sentences that restate the question.
2. Regardless of whether or not the care provider orders additional tests, what advice do you think he/she would offer this patient? Base your answer on what you know about chronic inflammation.

3. Based on your IL-6 test results and the patient information, do you think the care provider for Patient #2 would have reason to consider additional testing? Explain your answer. Please use complete sentences that restate the question.
4. Regardless of whether or not the care provider orders additional tests, what advice do you think he/she would offer this patient? Base your answer on what you know about chronic inflammation.

Complete the KQA anticipation chart (Summative assessment #1)

Students should now fill in the 3rd column of KQA chart

The End Product

(Summative assessment #2):

a) The Poster

b) The Poster Session for community (in this **mock** case, community will be members of the school community, which will represent the people attending the mock hospital education presentation)

POSTER AND POSTER PRESENTATION RUBRIC

CATEGORY	A HOME RUN (4 pts)	3rd base (3 pts)	2nd base (2 pts)	1st base (1 pt)	Try again (0 pts)
Coverage of the Topic IA: Basics of the Immune System --Innate immunity	Details on the poster capture the important information about the topic and increase the audience's understanding.	Details on the poster include important information but the audience may need more information to understand fully.	Details on the poster relate to the topic but are too general or incomplete. The audience needs more information to understand.	Details on the poster have little or nothing to do with this topic.	There is no information about this topic
Coverage of the Topic IB: Basics of the Immune System --Adaptive Immunity	Details on the poster capture the important information about the topic and increase the audience's understanding.	Details on the poster include important information but the audience may need more information to understand fully.	Details on the poster relate to the topic but are too general or incomplete. The audience needs more information to understand.	Details on the poster have little or nothing to do with this topic.	There is no information about this topic
Coverage of the Topic II: The Normal Inflammatory Process	Details on the poster capture the important information about the topic and increase the audience's understanding.	Details on the poster include important information but the audience may need more information to understand fully.	Details on the poster relate to the topic but are too general or incomplete. The audience needs more information to understand.	Details on the poster have little or nothing to do with this topic.	There is no information about this topic
Coverage of the Topic III: Chronic Inflammation	Details on the poster capture the important information about the topic and increase the audience's understanding.	Details on the poster include important information but the audience may need more information to understand fully.	Details on the poster relate to the topic but are too general or incomplete. The audience needs more information to understand.	Details on the poster have little or nothing to do with this topic.	There is no information about this topic
Coverage of the Topic IV: Dental infections and Chronic Inflammation	Details on the poster capture the important information about the topic and increase the audience's understanding.	Details on the poster include important information but the audience may need more information to understand fully.	Details on the poster relate to the topic but are too general or incomplete. The audience needs more information to understand.	Details on the poster have little or nothing to do with this topic.	There is no information about this topic
Coverage of the Topic V: Obesity and Chronic Inflammation	Details on the poster capture the important information about the topic and increase the audience's understanding.	Details on the poster include important information but the audience may need more information to understand fully.	Details on the poster relate to the topic but are too general or incomplete. The audience needs more information to understand.	Details on the poster have little or nothing to do with this topic.	There is no information about this topic
Coverage of the Topic VI: A measure of chronic inflammation: Determination of IL-6 by ELISA	Details on the poster capture the important information about the topic and increase the audience's understanding.	Details on the poster include important information but the audience may need more information to understand fully.	Details on the poster relate to the topic but are too general or incomplete. The audience needs more information to understand.	Details on the poster have little or nothing to do with this topic.	There is no information about this topic
Coverage of the Topic VII: Glossary/ Explanation of terms	Details on the poster capture the important information about the topic and increase the audience's understanding.	Details on the poster include important information but the audience may need more information to understand fully.	Details on the poster relate to the topic but are too general or incomplete. The audience needs more information to understand.	Details on the poster have little or nothing to do with this topic.	There is no information about this topic
Use of Graphics	All graphics are related to the topic and make it easier to understand.	All graphics are related to the topic and most make it easier to understand.	All graphics relate to the topic.	Graphics do not relate to the topic.	There are no graphics
Organization	Information is very organized with clear titles and subheadings.	Information is organized with titles and subheadings.	Information is organized, but titles and subheadings are missing or do not help the reader understand.	The information appears to be disorganized.	----------

Layout and Design	All information on the poster is in focus and can be easily viewed and identified from 6 ft. away.	Most of the information on the poster is in focus and the content easily viewed and identified from 6 ft. away.	Most of the information on the poster is in focus and the content is easily viewed and identified from 4 ft. away.	Much of the information on the poster is unclear or too small.	----------
Sources	All sources (information and graphics) are accurately documented. (MLA)	All sources (information and graphics) are accurately documented, but there are a few errors in the format. (MLA)	All sources (information and graphics) are documented, but information is incomplete or many are not in the desired format. (MLA)	Some sources are not accurately documented.	There is no documentation of sources
Mechanics	No grammatical, spelling or punctuation errors.	Almost no grammatical, spelling or punctuation errors.	Some grammatical, spelling, or punctuation errors, but they do not interfere with the reader's ability to understand	Many grammatical, spelling, or punctuation errors. Some interfere with the reader's ability to understand	----------
Presentation I: ability to explain and answer questions	When asked to explain poster contents, the presenter spoke clearly and distinctly and established eye contact with others.	The presenter spoke clearly most of the time and established some eye contact with others, but seemed hurried or too slow.	The presenter spoke unclearly most of the time, and/or established minimum eye contact with others. Explanation was confusing and/or seemed hurried or too slow.	The presenter spoke unclearly and/or did not establish eye contact with others. Others could not understand what the presenter was trying to convey	No attempt to explain to others
Presentation II: ability to answer reasonable questions about the information on the poster (not medical questions)	Presenter is able to answer all questions	Presenter is able to answer most questions	Presenter is able to answer some questions	Presenter is unable to answer most questions	Presenter cannot answer any questions
Collaboration	All members of the group contributed ideas in relatively equal amounts.	All members of the group contributed something to the chart.	Two members dominated or some members of the group did not contribute.	One member dominated or some members of the group did not contribute.	There were disputes and no collaboration

Name:_____ Date:_____

Student Worksheets

ELISA LAB: Semi-quantitative determination of Human IL-6

Preparation: Before we do the ELISA lab, you must become proficient at micropipetting skills and working with small quantities

http://www.biotech.iastate.edu/publications/lab_protocols/PipettorHampton.pdf

- Print out this page and complete the activity

ELISA LAB: Semi-quantitative determination of Human IL-6

In this laboratory activity, you will be working collaboratively in groups of 4 to determine the IL-6 level of 2 patients based on the levels in known standards.

- Be sure to follow safety protocol and any additional rules that your teacher reviews with you.
- Goggles, aprons and gloves are required at all times.
- As with all lab activities, no food or drink is permitted during this lab activity

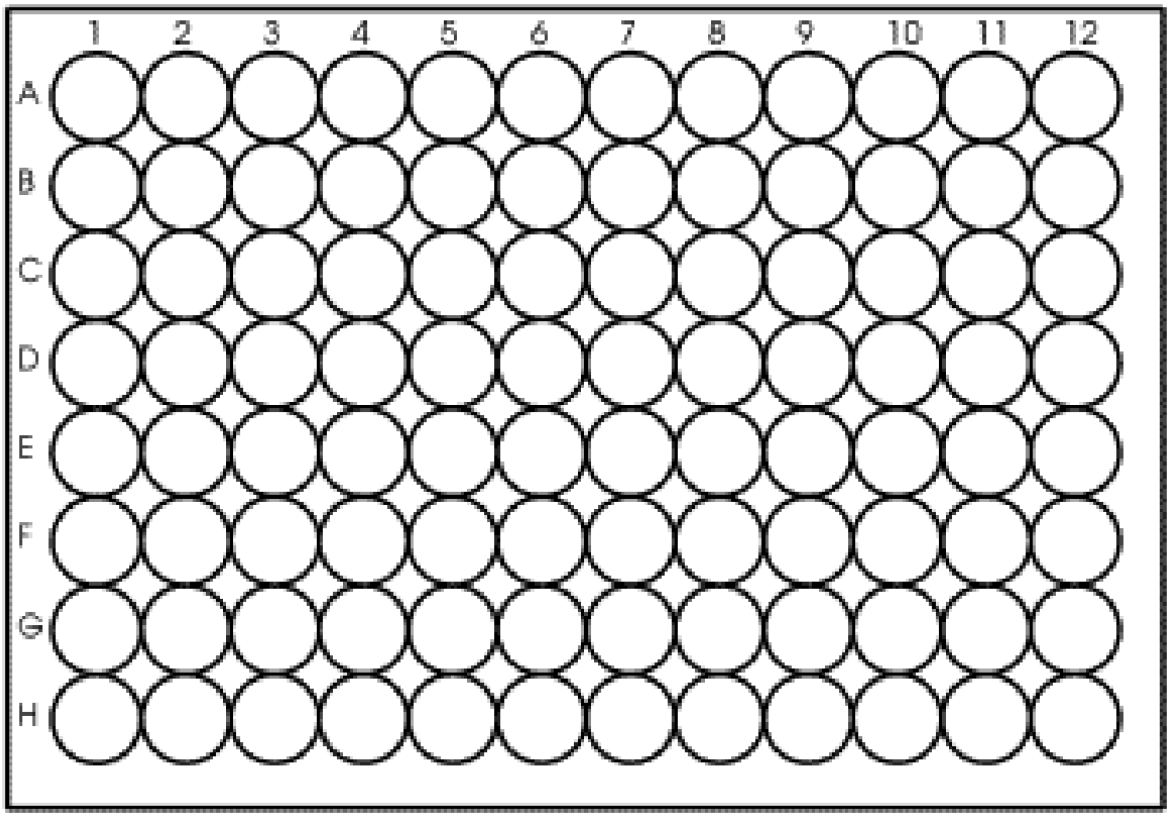
STUDENT WORKSTATION CHECKLIST #1

Check that your workstation contains each of the items on the list.

Item	Contents/volume	Number/amount
ELISA plates		4 (1 per student)
ELISA plate sealers		4 (1 per student)
micropipetters	Capable of delivering 100, 200 and 250 µL	1-4 (students can share, but ideally each student would have their own)
micropipetter tips		A supply of at least 50 tips
sink or wash bucket		1
Capture antibody		1 microcentrifuge tube
Paper toweling		1 roll

1. Label your ELISA plate template below as follows:

Well	Contents	Write this on template
A-1	**IL-6 standard 200 pg/mL**	**200**
B-1	**IL-6 standard 100 pg/mL**	**100**
C-1	**IL-6 standard 50 pg/mL**	**50**
D-1	**IL-6 standard 25 pg/mL**	**25**
E-1	**IL-6 standard 12.5 pg/mL**	**12.5**
F-1	**1x ELISA diluent (Blank)**	**0 - Blank**
G-1		**Patient 1**
H-1		**Patient 2**



2. Coat wells A-H of the ELISA plate with the Capture Antibody:

- Add 100µL of the Capture Antibody to each well.
- Seal the plate with the plate sealer.
- Incubate in refrigerator at 4C overnight.

STUDENT WORKSTATION CHECKLIST #2

Check that your workstation contains each of the items on the list.

Item	Contents/volume	Number/amount
micropipetters	**Capable of delivering 100, 200 and 250 µL**	**1-4 (students can share, but ideally each student would have their own)**
1x Diluent		**1 microcentrifuge tube**
micropipetter tips		**A supply of at least 50 tips**
sink or wash bucket		**1**
ELISA wash buffer	**500 mL**	**1 wash bottle**
Top IL-6 standsrd		**1 microcentrifuge tube**
Microcentrifuge tubes (to prepare serial dilutions of standard)		**20 (5 per student)**
Paper toweling		**1 roll**

1. Wash and block:

- Remove the liquid from the wells by "flick method" (demonstrated in the video/animation)
  SECURELY hold the ELISA plate in the palm of your hand flat then pretend that you have a baseball (or fly swatter) and quickly snap your arm propelling your hand towards the sink. Blotting is best done with some force. Holding the plate in your hand, bring it down to "hit" the stack of napkins, but not so hard as to break the plate
  It would be helpful for you to practice with plates and water.
  Do not allow the wells to dry out. Proceed promptly to the wash steps.

Wash 3 times with Wash Buffer (demonstrated in the video/animation)

- Fill each well using a squirt bottle.
- Allow wells to soak in Wash Buffer for approximately 1 min during each wash step.
- Blot plate on a thick layer of absorbent paper to remove any residual buffer

Block wells by adding 200µL 1X ELISA Diluent to each well

- Seal the plate with the plate sealer.
- Incubate in the refrigerator at 4°C overnight

2. In the meantime, prepare the standards

Prepare 4 serial dilutions of the IL-6 "top standard" (200 pg/mL) in Eppendorf tubes as follows:

- Label Eppendorf tubes A(200), B(100), C(50), D(25), E(12.5).
- Add 250 µL of ELISA Diluent to Eppendorf tubes B-E.
- Add 500 µL of the "top standard" to tube A.
- Remove 250 µL from tube A and transfer to tube B and mix by pipeting up and down. Tube B now contains the 100 pg/mL standard.
- Remove 250 µL from tube B and transfer to tube C and mix by pipeting up and down. This tube now contains the 50 pg/mL standard.
- Remove 250 µL from tube C and transfer to tube D and mix by pipeting up and down.This tube now contains the 25 pg/mL standard.
- Remove 250 µL from tube D and transfer to tube E and mix by pipeting up and down. This tube now contains the 12.5 pg/mL standard.
- Remove 250 µL from tube E and dispose of it so that all tubes contain 250 µL total**.**
- Incubate in the refrigerator at 4°C overnight.

STUDENT WORKSTATION CHECKLIST #3

Check that your workstation contains each of the items on the list.

ELISA plates		**4 (1 per student)**
Item	**Contents/volume**	**Number/amount**
micropipetters	**Capable of delivering 100, 200 and 250 µL**	**1-4 (students can share, but ideally each student would have their own)**
micropipetter tips		**A supply of at least 50 tips**
sink or wash bucket		**1**
Standards that were prepared on Day 2		**Each student has their own set they prepared**
Patient sample #1		**1 microcentrifuge tube, aliquot**
Patient sample #2		**1 microcentrifuge tube, aliquot**
1x Diluent		**1 conical test tube,**
Paper toweling		**1 roll**
Wash buffer		**1 - 500 mL in squeeze wash bottle**

Add STANDARDS, Patient SAMPLES and BLANKS to well.

- Remove the liquid from the wells by flicking.
- Add 100 µL of each of the 5 standards prepared yesterday to the appropriate wells according to the Well Template.
- Add 100 µL of the Elisa Diluent to serve as blanks to the appropriate wells according to your Well Template.
- Add 100µL of each of your patient samples to the appropriate wells according to the Well Template.
- Seal the plate with the plate sealer.
- Incubate at 4C overnight.

STUDENT WORKSTATION CHECKLIST #4

Check that your workstation contains each of the items on the list.

Item	Contents/volume	Number/amount
micropipetters	**Capable of delivering 100, 200 and 250 µL**	**1-4 (students can share, but ideally each student would have their own)**
micropipetter tips		**A supply of at least 50 tips**
sink or wash bucket		**1**
Capture antibody		**1 microcentrifuge tube aliquot**
Enzyme		**1 microcentrifuge tube aliquot**
Substrate		**1 microcentrifuge tube aliquot**
STOP solution		**1 microcentrifuge tube aliquot**
Paper toweling		**1 roll**
Wash buffer		**1 - 500 mL in squeeze wash bottle**

1. Add the DETECTION ANTIBODY

- ☐ Remove the liquid from the wells by flicking.
- ☐ "Flick and wash" as on Day 2 in steps 1a and b. Repeat for a total of 3 washes.
- ☐ Add 100 µL/well of the Detection Antibody to each well.
- ☐ Seal the plate with the plate sealer.
- ☐ Incubate at room temperature 1 hr.

2. Add the ENZYME

- ☐ "Flick and wash" as on Day 2 in steps 1a and b. Repeat for a total of 3 washes.
- ☐ Add 100 µL/well of the Enzyme.
- ☐ Seal the plate with the plate sealer.
- ☐ Incubate at room temp 30 min.

3. Add the SUBSTRATE and the STOP solution

- ☐ "Flick and wash" as on Day 2 in steps 1a and b. Repeat for a total of 5 washes
- ☐ Add 100 µL of Substrate (1X TMB) to each well.
- ☐ Watch carefully now for the first hint of blue color in well C1.
- ☐ Quickly, but steadily add 100 µL of STOP Solution to each well, beginning with A1 and following the same order that you have been doing.

It is important to add the **STOP** solution in the same order as the substrate and at the same steady pace.

4. Determine IL-6 concentration

- ☐ Determine the approximate range of IL-6 in your Patient samples by visually comparing them to the standards.
- ☐ Assign a range based on the color intensity. For example, if the color intensity of Patient Sample 1 falls in between that of Std #2 and Std #3, you would determine the IL-6 value for Patient 1 to be between 50 – 100 pg/µL.

	Known IL-6 level, pg/mL	Approximate IL-6 range (visually compared to standards)
Std #1	**200**	**----------**
Std #2	**100**	**----------**
Std #3	**50**	**----------**
Std #4	**25**	**----------**
Std #5	**12.5**	**----------**
Blank	**0**	**----------**
Patient #1	**--------**	
Patient #2	**--------**	

In this laboratory, considering limitations of this test, we will consider any detectable level of IL-6 to be an *elevated level*. The normal range of IL-6, with this test, is below the detectable levels, or in this case, 0 pg/mL. Elevated levels of IL-6 can be due to a number of reasons, one of which may be the presence of a chronic inflammatory state. (In this case, the patient's care provider may choose to investigate further and order more tests. It is important to realize that a single test does not always provide the complete story!)

You are given the following information about each patient:
Patient #1 is 28 years old. Patient has recently reported constant "excruciating" pain from area in mouth "throughout lower jaw." Area seems tender to the touch. Patient reports recurring "toothaches" in that area during the past "year or so –usually flares up about every six weeks." At those times, patient self-medicates with over the counter products –"usually Tylenol or Advil, which provide relief, but this time, those medications have not been effective in relieving the pain at all."
Visual examination reveals swelling and redness of the lower right jaw and general poor dental hygiene. Patient admits that friends and family often remark that he has "bad breath." Patient reports last dental visit 10 years ago.

Patient #2 is 18 years old and is 190 lbs. Normal weight for patient of this age, height and build is 140 lbs. Patient complains of being "tired all the time and hungry a lot."
When questioned about diet, patient admits to poor nutritive choices

- eats very few fruits, vegetables and whole grains
- chooses mostly high fat, highly processed foods
- drinks no water
- usually soda and energy drinks
- often eats at fast food restaurants and usually orders the "super-size" option.

Conclusion Questions:

1. Based on your IL-6 test results and the patient information, do you think the care provider for Patient #1 would have reason to consider additional testing? Explain your answer. Please use complete sentences that restate the question.

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2. Regardless of whether or not the care provider orders additional tests, what advice do you think he/she would offer this patient? Base your answer on what you know about chronic inflammation.

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3. Based on your IL-6 test results and the patient information, do you think the care provider for Patient #2 would have reason to consider additional testing? Explain your answer. Please use complete sentences that restate the question.

4. Regardless of whether or not the care provider orders additional tests, what advice do you think he/she would offer this patient? Base your answer on what you know about chronic inflammation.

The Poster and Presentation

- Prepare the poster for Mountain Valley Regional Medical Center.
- Follow the Poster and Poster Presentation Rubric that you have been given very carefully.
- Make sure that you cover each graded item on the rubric:
    - Coverage of the Topic IA: Basics of the Immune System - Innate immunity
    - Coverage of the Topic IB: Basics of the Immune System - Adaptive Immunity
    - Coverage of the Topic II: The Normal Inflammatory Process
    - Coverage of the Topic III: Chronic Inflammation
    - Coverage of the Topic IV: Dental infections and Chronic Inflammation
    - Coverage of the Topic V: Obesity and Chronic Inflammation
    - Coverage of the Topic VI: A measure of chronic inflammation: Determination of IL-6 by ELISA
    - Coverage of the Topic VII: Glossary/ Explanation of terms
    - Use of Graphics
    - Organization
    - Layout and Design
    - Sources
    - Mechanics
    - Presentation I: ability to explain and answer questions
    - Presentation II: ability to answer reasonable questions about the information on the poster (not medical questions)
    - Collaboration