Indirect, Competitive Enzyme-Linked Immunosorbent Assay Determination of Secretory Immunoglobulin A Levels in Saliva.

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The research was funded by the American Association of Immunologists and supported by UMDNJ.

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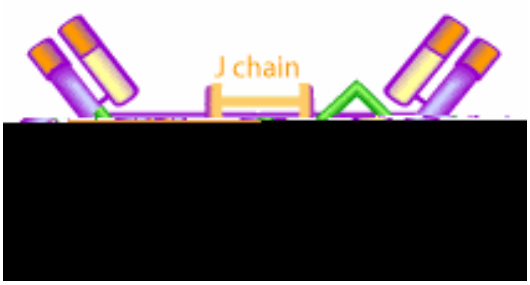
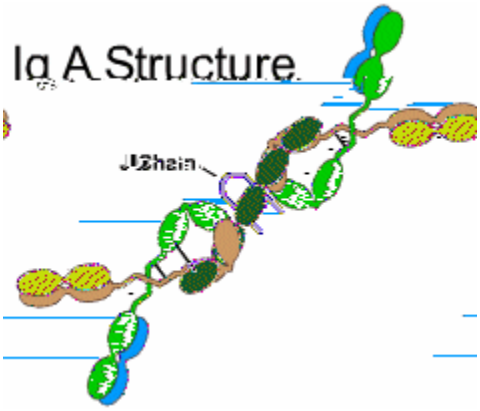
Enzyme-Linked Immunosorbent Assay determination of immunoglobulin A levels in saliva.





Overview

curriculum, especially in schools where there are small learning communities or clusters. Within the biology or health curriculum, this activity will fall under the immune system. The students will learn the role of antibodies in protecting against infections. The students will also learn how to perform the ELISA protocol and appreciate its importance in immunology.

The teacher should contact the local Board of Education to get approval for the use of saliva in a classroom setting.

Science Background

Salivary Immunoglobulin A

after acute exercise. The effects of exercise on s-IgA are therefore still under intense study.

IgA deficiency is quite common, with approximately one in every 500 people (some studies show 1 in 700) people presenting a deficiency of IgA. Most of these people appear healthy and may never know that they have selective IgA deficiency. Conversely, there are people with IgA deficiency that have severe illnesses. It is yet unknown why some people with IgA deficiency are very sick while others are not. It is therefore very important that du

5. Enzyme-linked secondary antibody that is specific to the primary antibody is added.
6. The plate is washed so that the unbound antibody-enzyme conjugates are removed.
7. A chemical is added that is conv

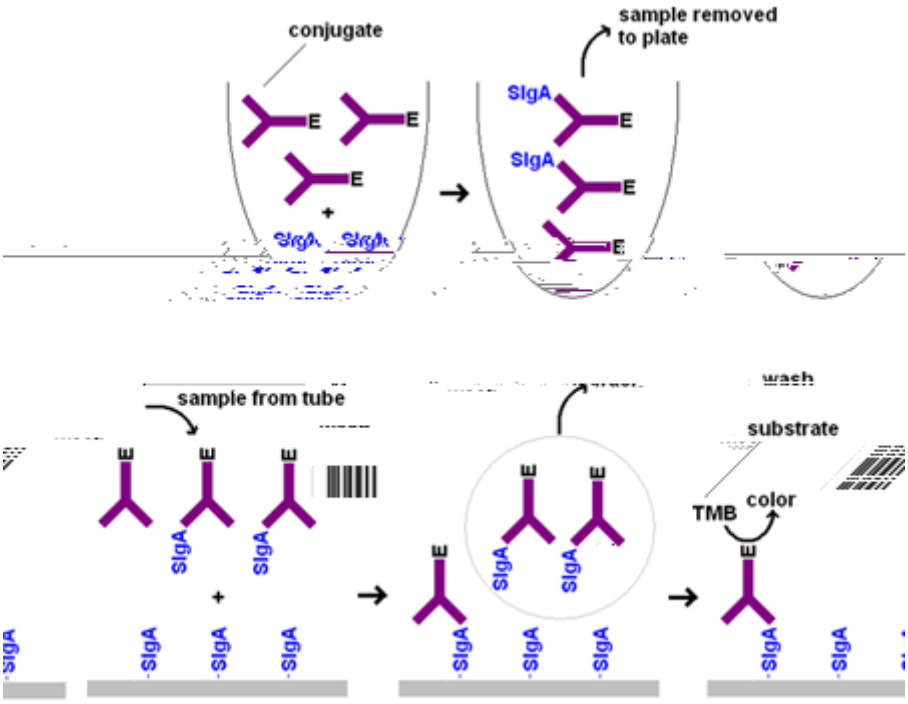


Fig 2. Indirect Competitive Assay for SIgA. Adapted from www.salimetrics.com.

Learning Objectives

Students will research and describe the importance of saliva in the first line of defense against pathogens.

Students will describe the theory and process of ELISA- an important technique in immunology.

Students will perform serial dilutions and construct dilution curves that they will use to determine concentrations of unknowns

Students will assemble a simple spectrophotometer for schools that do not have a standard spectrophotometer.

Students will identify the differences in salivary IgA concentrations between groups

Students will apply knowledge gained in the first lab on simple dilutions to determine the SIgA concentrations between different groups in the second lab.

New Jersey Core Curriculum Content Standards: 5.1, 5.3 and 5.5.

Time Requirements

Approximately 3 double period classes will be needed to complete all the lessons.

Day 1. - Introduce the lesson and give a rationale for the consequent labs. The teacher will also give a background on dilutions, ELISA and Immunoglobulin A. In the second half of the class the students will play the secretory IgA game.

Day 2- Assemble the simple spectrometer and perform the simple dilutions lab.

Day 3- Students will perform the sIgA lab.

Advance preparations:

SIgA classroom activity

Prepare 50 immune system questions on index cards. On the back of 25 of these questions write the word bacteria and on the other 25 write the word virus.

Make the following cards:

On 4 index cards write *SIgA*
On 1 write *IgA secretory receptor*
On 1 index card write *enzyme*
On 1 *bacteria*
And on 1 *virus*

On each card write what each does. These cards will be taped onto the shirts of respective group members during the activity.

Prepare a large chart-size construction paper or cloth to represent the respiratory membrane. This will be taped onto the wall.

Prepare a points record chart where each group's points will be recorded and also taped to the wall.

Dilution lab:

Unknowns should be made ahead of time with student input. Examples of how the unknowns can be made:

Collect samples of soft drinks from stores, movie theatres and restaurants- with and without ice. Label each sample clearly. These will serve as the unknowns. This is the unknown source that I used with my students.

Alternatively add 100ml of soft drink and 50g of crushed ice into different containers, take 10ml samples at set time intervals.

Another option is to add differing amounts of ice to set amount of soft drink and heat to melt the ice.

SIgA ELISA lab:

1. The teacher should find an immunology laboratory for steps 7-10 of this lab. A local university or college with an immunology department should have the necessary equipment. This arrangement will need to be made well in advance of starting the lab, preferably at the beginning of the academic year.
2. Students should be able to use a pipette before the lab.
3. Determine the plate layout. The kit I recommend here can hold up to 76 samples. It is best to use one kit per class as the standards are limiting but with good organization, one kit can be used for two or more classes to cut on costs. The cost for one sIgA ELISA kit is about $320 (The kit can be purchased from salimetrics Inc., http://www.salimetrics.com/products).
4. Go over the procedure and complete the labels for the tubes- this can be done with student participation.
5. Explain to the students that each

6. The procedure is long, so discuss it with the students prior to the lab.
7. Select students that have mastered lab1 on simple dilutions to serially dilute the standards as a demonstration to the class. Prior to the lab- go over the procedure with these students.

Setting up Spectrophotometer

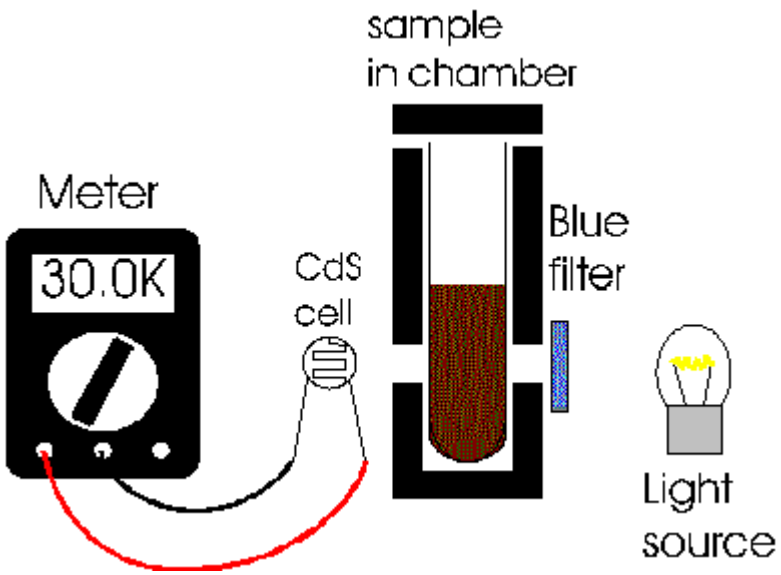


Fig. 3. How to set up a simple spectrophotometer. Adapted from www.stanford.edu/groups/Urchin/simple.htm

Reference:

Sea urchin embryology- simple dilutions.www.stanford.edu/groups/Urchin/simple.htm

Salivary IgA ELISA lab
For a class of 30 with 15 groups
Saliva collection

30 Plastic drinking straws- regular size.
15 Scissors
30 Cryovials: polypropylene- 2ml. (*Microcentrifuge tubes will do or any tube that can hold 2mL of solution and can be capped)*)
Labels
Gloves for each student
Safety goggles for each student
Apron for each student.
One calculator per group.

One 96 Tw 0.46 0 Td [( )-549im

1. Make cards to represent saliva, two molecules of SIgA showing a binding site (you will have four students do this as this is a dimeric protein), 1 bacteria (with the same binding site that corresponds to the SIgA), 1 virus, 1 IgA secretory receptor and one enzyme . On the backs of these cards write down what each does. The cards should be color coded to make it easier for students to recognize them. Additionally, the teacher will have prepared 50 questions on immune system and written them on index cards, 25 questions labeled virus and 25 labeled bacteria. Students can be involved in the preparation of these questions.

Sample questions:

a) Give two examples of organs that are involved in the immune response.
b) What are the two types of T cells?
c) Explain the difference in structure between IgA and IgM
d) In what parts of the body is IgA found?
e) What cell type makes antibodies?

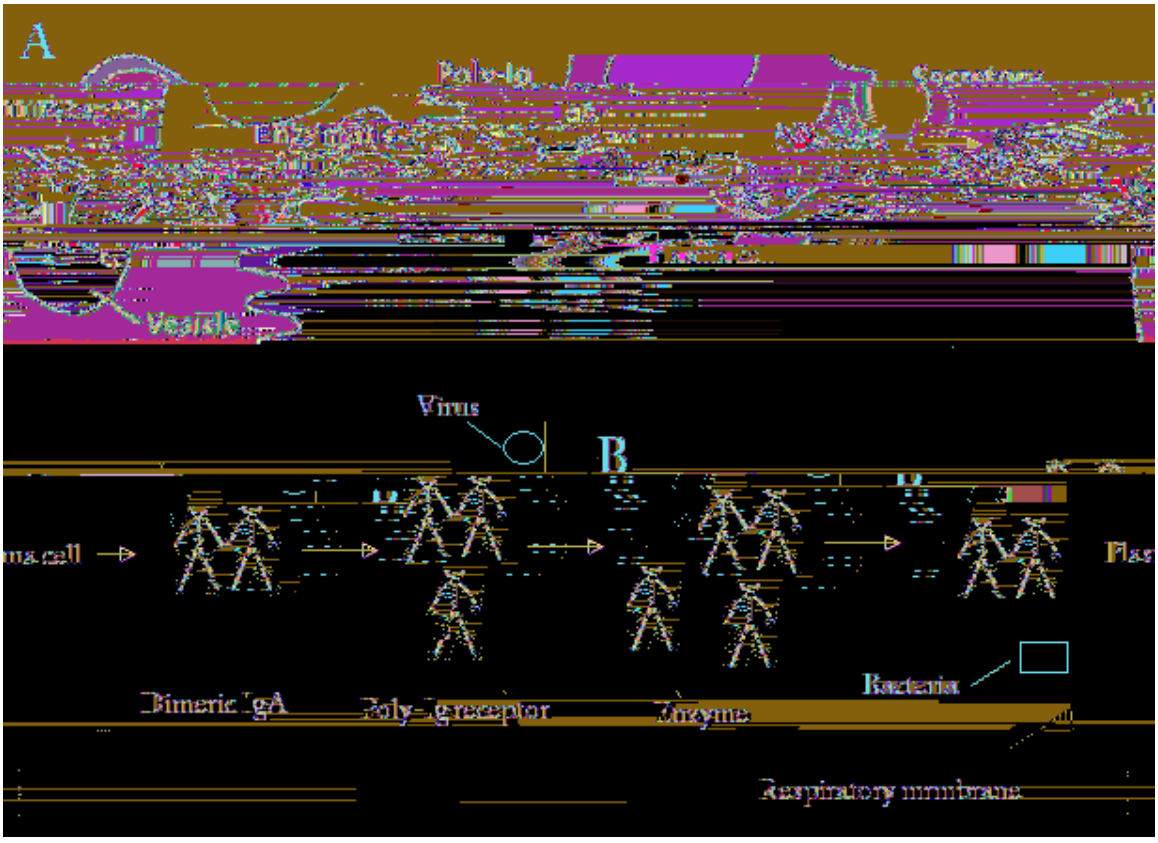


Fig. 4. The process of sIgA synthesis and secretion. A). After synthesis by plasma cells, dimeric IgA passes through the mucosal epithelial membrane in a poly-Ig receptor mediated manner. sIgA is released into the respiratory

pathway. One group will role play the pathway and answer questions to protect the respiratory membrane while an opposing group will ask the questions and stick bacteria or virus onto the membrane when questions are answered incorrectly.

Students will be divided into groups of 6. Each group will take turns as either group A or B. Each member of each group will take a role as one of the following (See fig. 4):

- Bacteria- I adhere to the mucosal surface and cause an infection
- Virus- I pass through the mucosa and get into the epithelial cells of the respiratory surface, multiply and kill the cells.
- IgA secretory receptor- I am responsible for transportation of IgA from the lymphoid tissue where it is made by plasma cells to the lumen of the respiratory tract. I remain attached to the IgA until the enzyme cleaves me off
- First SIgA – I bind to the bacteria and prevent them from binding to mucosal surface
- Second SIgA- I bind the virus and prevent them from getting through the mucosa, multiplying and killing the cells
- Enzyme- I cleave off the SIgA receptor so that the SIgA is released into the respiratory lumen.

2. Have one large piece of cloth or paper to represent the respiratory membrane. This should be taped onto the wall
3. There will be two opposing groups (For larger class sizes, devide students into groups of six)- each consisting of:
    a) Two students to represent dimeric sIgA
    b) 1 student to represent poly-Ig receptor.
    c) 1 student to represent the enzyme.
    d) 1 student to represent virus
    e) 1 student to represent bacteria
4. To start the activity, group B's SIgA, enzyme and receptor will go outside the classroom door. The two pairs of students (each pair holding hands to represent the dimeric nature of IgA) representing Salivary IgA will then enter the class through the door while attached to the IgA receptor. The enzyme will follow behind. Each student will read their card to the class.
5. Once inside the class, the student representing the enzyme will separate the receptor from the IgA pair. The IgA pair will move to the membrane and stand guard.
6. Group A will then be provided with 10 immune system questions to ask group B.
7. Group B will have 1 minute to answer each question. Every time a wrong answer is given, the card that contains that question is stuck onto the membrane behind the IgA and represents either a virus or bacteria. Six or more stuck cards represent an infection if more than half of them are bacteria or epithelial cell death if more than half are viruses.

8. The groups will then take turns until each group has played the game as either group A or B

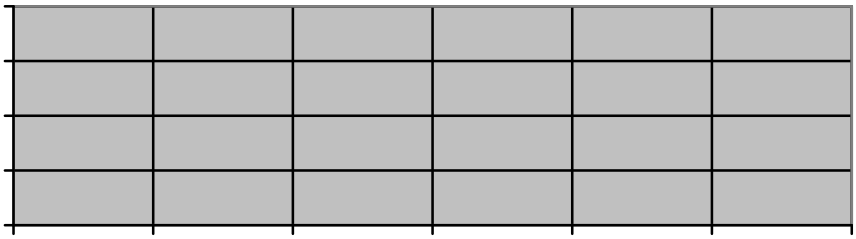
Background:
The ability to make dilutions is crucial in biology and chemistry. Students will need to master the skills in this lab to easily grasp the concepts in the salivary IgA ELISA experiment.
In this experiment, dilutions will be related to the real world.
SCENARIO- You have purchased a soft drink at the restaurant. Is it at the right concentration? Has the soft drink been "watered down" to save money. When the ice melts- how much more dilute is the soft drink then?
Using the soft drink as the material of interest, students will perform a simple linear dilution and construct a standard curve from which they will quantify unknown samples. For schools without a spectrophotometer, a simple one can be constructed using a light sensor, light source, filter and a meter. Soft drink absorbs light in the blue end of the spectrum. Therefore a filter that primarily lets blue light through is used.

Standard series:

Tube number	1	2	3	4	5	6
% soft drink	100	80	60	40	20	0
Ml of soft drink	10	8	6	4	2	0
Ml of water	0	2	4	6	8	10
Total liquid	10	10	10	10	10	10

Anticipated Results:

Plot absorbance readings readings vs. concentration as shown in the example below. Remember that the student standard curves may appear quite different from the one shown here.



Day 3
Secretory IgA-specific ELISA lab

The principle of the IgA- specific ELISA test
A constant amount of goat anti-human sIgA conjugated to horseradish peroxidase is added to tubes containing specific dilutions of standards or saliva. The antibody-conjugate binds to the SIgA in the standard or saliva samples. The amount of free antibody remaining is inversely proportional to the amount of SIgA present. After incubation and mixing, an equal solution from each tube is added in duplicate, to microtiter plate coated with human sIgA. The free or unbound antibody conjugate binds to the SIgA on the plate. After incubation, unbound components are washed away. Bound conjugate is measured by the reaction of peroxidase enzyme on the substrate TMB. This reaction produces a blue color. A yellow color is formed after stopping the reaction. Optical density is read on a standard plate reader at 450nm. The amount of peroxidase is inversely proportional to the amount of SIgA present in the sample[4].

Methods
Collecting whole saliva samples by passive drool from human subjects

Things to have the students avoid:

Brushing teeth within 1 hr prior to collection (may lead to gum bleeds with consequent contamination of saliva with serum IgA)
Using salivary stimulants: chewing gum, lemon drops, granulated sugar, drink crystals*.
Consuming a major meal within 1 hr prior to collection*
Consuming acidic or high sugar foods within 20 min of collection*.

* *May lower saliva pH and increase bacterial growth.*

Instruct students to:

1. Rinse mouth with water 10 min prior to sample collection.
2. Record time of day sample is collected
3. Record time taken for each donor to accumulate 1ml of saliva in the cryovial/microcentrifuge tube.

Prior to saliva collection:

Cut plastic drinking straws into 2-inch (5cm) pieces.
Make a 1ml mark on each. This can be done by adding 1 ml of water to the cryovial using a pipette.
Give each student 1 straw piece and (1) cryovial.
Have students rinse their mouth with water 10 minutes prior to collection.

Collecting Saliva:

1. Students will work in pairs and the saliva from each pair will eventually be mixed.
2. Students will use permanent markers to label their tubes with their two initials.
3. Instruct students to imagine eating their favorite food and allow saliva to pool in the mouth.
4. With head tilted forward, student should drool down the straw and collect saliva into the cryovial (*It is normal for saliva to form).*
5. Repeat as often as necessary until 1 ml of saliva is collected, less the foam.
6. Each group member will record the time he or she takes to collect 1mL of saliva.
7. If student's mouth is dry, instruct them to gently chew on the end of the straw. This will stimulate saliva production.
8. After collecting the samples, students will use 1000ul pipettes to transfer 1mL saliva from one partner's vial into the other's vial. The students will use the pipette to thoroughly mix the saliva while carefully avoiding foam formation. The students will label the samples using their two last name initials (one initial from each student in group, see the student instructions on page 38). If pipettes are limiting, the teacher may carry out the transfer and mixing as a demonstration.
9. Students will close the vials tight and bind the two vials with an elastic band.
10. Use samples immediately after collection or keep cold temporarily at 4°C. When samples remain at room temperature longer than a few hours there is opportunity for bacterial growth that can invalidate the assay. While carrying the samples to the immunology lab for reading, place the saliva tubes in an ice box with ice.
11. The rest of the steps will take place in an immunology laboratory as indicated on the protocol below.

Salivary IgA Quantitation Protocol

Mix all reagents before use. You can test upto 76 samples on one plate. If possible, combine classes so you can save on the materials. Steps 7, 8, 9 and 10 of the ELISA process should be done in a laboratory with the appropriate equipment. It will be necessary for the teacher to find an immunology research laboratory and form a working relationship with them. These arrangements should be done way prior to planning the experiment and the dates and times the lab is available included in the experiment plan. The teacher may make arrangements to bring a group of students to the immunology lab. This will introduce students to actual immunology lab procedures.

Calculate flow Rate for each sample.

The teacher will make a chart of the data tables in this section and paste these charts on the classroom wall. The teacher will record the average time in seconds and use numbers for sample ID. It is imperative that you not use student names or initials as sample ID. When chart is complete, each student will copy the data tables (the data tables are provided in the student section) and complete the calculations as detailed in the student section.

Flow Rate = Saliva volume (mL)/Time (Min)

Sample ID	Volume (mL)	Time (seconds)- average of two students	Time (minutes)- average of two students	Flow rate (mL/min)- average of two students
	1mL			
	1mL			
	1mL			
	1mL			
	1mL			
	1mL			
	1mL			
	1mL			
	1mL			
	1mL			
	1mL			
	1mL			
	1mL			
	1mL			
	1mL			
	1mL			
	1mL			
	1mL			
	1mL			
	1mL			

Table 2.

ELISA:

The quantities of reagents described here is for one row of standards and samples. Adjust the quantities to fit the number of samples. Double the reagents for the standard to end up with a duplicate row.

1 Determine your plate layout. Here is a sample layout. This layout can hold samples from more than 140 student pairs. If you have only three classes, then Rows A and B hold the standards, C and D samples from class 1, E and F samples from class 2, G and H samples from class 3.

	1	2	3	4	5	6	7	8	9	10	11	12
A	600.000	200.000	66.700	22.200	7.400	2.500	0.000	0.000	blank			
B	600.000	200.000	66.700	22.200	7.400	2.500	0.000	0.000	blank			
C	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Low control		

Table 3.

2 Keep the desired number of wells in the strip folder and place remaining strips back in the pouch. Store pouch at 2-8 C.

3 Label five microcentrifuge pouches 2-6 and pipette 30ul of 1X SIgA diluent in each tube. Place 15ul of the 600ug/ml standard into tube 1. Serially dilute the standard 3X by adding 15ul from tube 1 into tube 2. Mix well and change pipette tips. Remove 15ul (30ul for duplicates) from tube 2 into tube 3. Mix well. Continue for tubes 4, 5 and 6. The final concentrations for tubes 1-6 are respectively, 600ug/ml, 200ug/ml, 66.7ug/ml, 22.2 ug/ml, 7.4ug/ml and 2.5 ug/ml. Pipette 3 ml of 1X SIgA diluent into a tube (*Scale down proportionately if not using entire plate*). Set aside for step 6.

4 Transfer saliva from each pair of students into one 2ml microcentrifuge tube. Mix thoroughly with a pipette or vortex. Transfer 1ml of this mixture into a new microcentrifuge tube and label.

5 Label 1 small tube with the identity of each saliva sample (*Do not pre-dilute controls 5X*). With a repeater pipette, add 100ul of 1X SIgA diluent into each tube. Pipette 25ul of saliva into the appropriate tube

6 Label one 12 X 75 mm snap-cap tube for each standard, control, and unknown sample, and one tube for the zero value. Using a repeater pipette add 4ml of 1X SIgA diluent to each tube. Add 10ul of standard (from step 3), control, or diluted unknown saliva sample (from step 4) to the appropriate tube. Add 10ul of 1X SIgA diluent to the zero tube.

7 Dilute the antibody-enzyme conjugate 1:120 by adding 25 ul of the conjugate to the 3ml of 1X SIgA diluent prepared in step 3. Scale down proportionately if not using entire plate). Mix well and pipette 50ul of the diluted antibody-enzyme conjugate to all tubes using a repeater pipette. Gently mix each tube by inversion and incubate for 90 minutes at room temperature.

8 Gently mix each tube by inversion again and add 50ul of solution from step 6 to the microtiter plate according to your template. Add 50ul of 1X SIgA diluent to the NSB wells. Seal plate and incubate at room temperature with continual mixing at 400 rpm for 90 minutes *(60-90 minutes is fine).*

9 Wash the plate 6 times with 300ul 1X wash buffer in each well.

10 Add 50ul TMB solution to each well with a multichannel pipette.

11 Mix on a plate rotator for 5 minutes at 500rpm *(not very necessary*) and incubate the plate in the dark at room temperature for an additional 40 minutes. *Though the protocol suggests 40 minutes, I found 2 minutes to be adequate. Incubating for more than 10 minutes led to color change that was too bright for the plate reader available to me (Tecan, GENios model).*

12 Add 50ul of stop solution with a multichannel pipette. Mix on a plate rotator for 3 min at 500rpm (or tap to mix). Be sure all wells have turned yellow. Read plate in a plate reader at 450nm. Read plate within 10 minutes of adding stop solution..

<u>Calculations:</u>

The students will perform these calculations with the teacher's guidance. The questions and tables are also provided in the student sections.

1. Compute the average OD for all duplicate wells
2. Subtract the average OD for the NSB wells form the average OD of the zero, standards, controls and unknowns.
3. Calculate the percent bound for each standard, control and unknown by dividing the average OD (B) by the average OD for the zero (Bo).
4. Determine the concentration for the control and unknown by interpolation
5. Multiply concentrations of unknown saliva samples by 5 to obtain the final concentration of SIgA in ug/ml.

Wells	Sample ID	Average OD	B	B/Bo	SIgA (ug/ml)

Table 4.

Corrected SIgA= absolute SIgA (ug/mL) x flowrate (mL/min)

Sample ID	Absolute SIgA (ug/mL)	Flow rate (mL/min)	Corrected SIgA (ug/min)

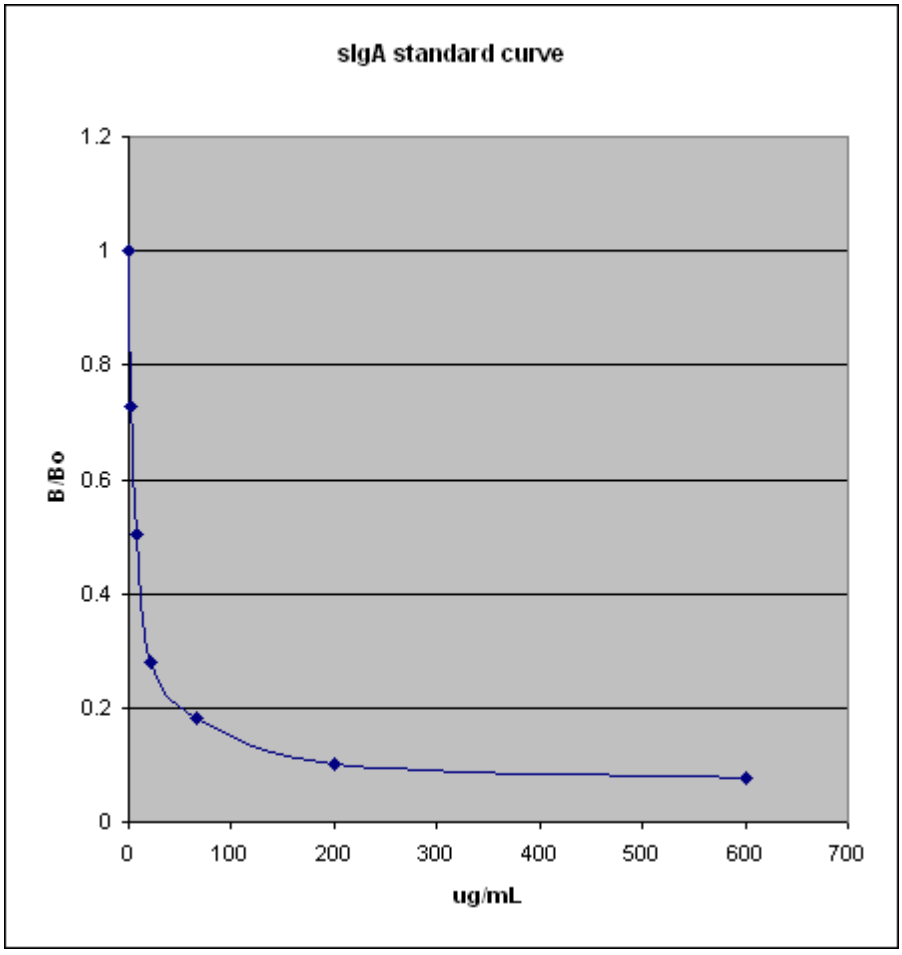
Table 5.

Sample data and results as obtained by this author

Name of Assay: sIgA quantitation ELISA091408
Incubation: 90 min
Date performed: 91408
Performed by: David Mwangi

Plate layout

	1	2	3	4	5	6	7	8	9	10	11
A	600.000	200.000	66.700	22.200	7.400	2.500	0.000	0.000	blank		


sIgA standard curve
B/Bo
1.2
1
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
600
700
ug/mL

Corrected sIgA amounts (ug/Min) = Absolute sIgA (ug/mL)x Flow rate (mL/Min)

Sample ID	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6	Donor 7	Donor 8	Donor 9	Low control	High control
sIgA	30	15	20	28	50	7	13	14	15	15	45
x5-Abs	150	75	100	140	250	35	65	70	75	75	225
FR	1.43	0.59	2.5	0.1	0.5	0.26	0.4	7.14	0.42		
Corr. sIgA	214.5	44.25	250	14	125	9.1	26	499.8	31.5	15	225

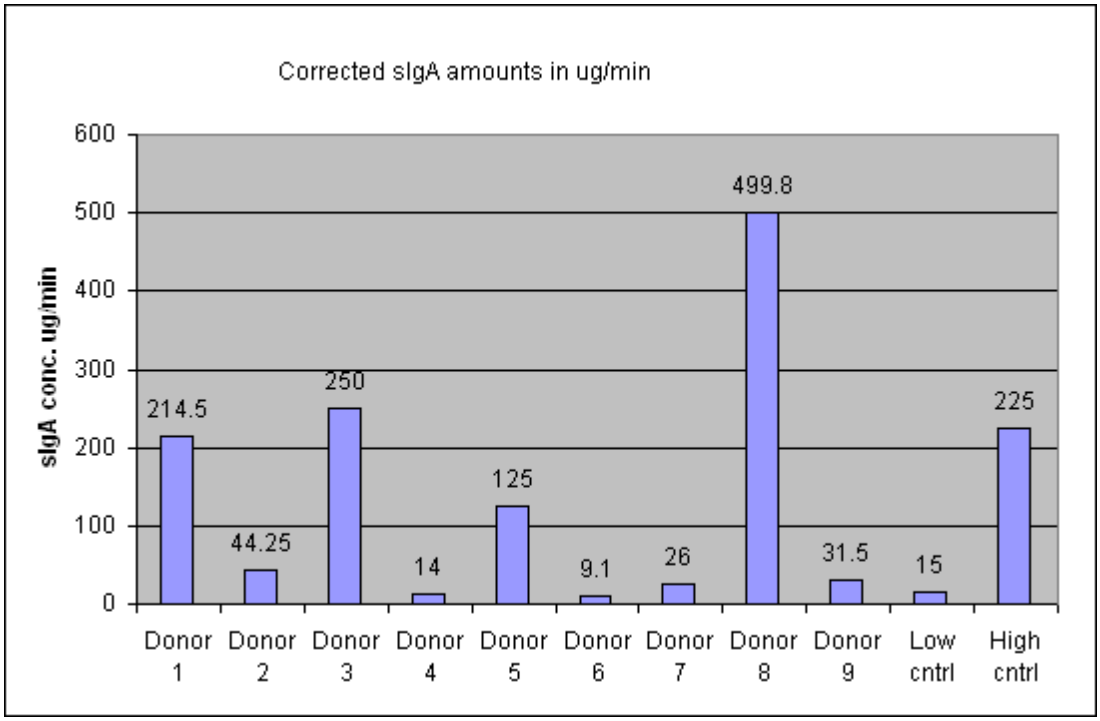


Figure 6.

Student Section

Rationale

Why is saliva important in the body's defense against pathogens?

Most people do not associate saliva with the immune system. However, saliva and mucosal secretions form an important first line of defense against pathogens invading the mouth. Saliva provides a mechanical washing effect to protect the oral mucous membrane, while IgA prevents viral replication and bacterial attachment to the mucosal surfaces. One of the most widely used techniques in immunology is ELISA. In this lab you will determine the quantity of IgA in your own saliva using ELISA. You will first play a game in which you will learn the path taken by IgA from its synthesis to the oral cavity. Then, you will complete two labs. In the first lab, you will become familiar with simple dilutions. You will use this skill in the second lab where you will determine the quantity of IgA in a saliva mixture. You will also complete a lab report and a research paper at the conclusion of this unit.

Background Information

Salivary IgA

Salivary IgA is an antibody found in saliva, in the gastrointestinal tract and in mucus secretions throughout the body. The major function of IgA is to prevent bacteria from binding to the mucous membrane and to prevent viruses from multiplying and killing membrane cells. It is estimated that in a normal adult, IgA constitutes 60%-70% of the total output of antibodies. The IgA form most common in saliva is the dimeric secretory IgA. IgA is made by mature B cells in lymph organs and transported to the oral membrane via a receptor. The IgA is then shed into the oral cavity after the receptor is cleaved by an enzyme. It is here that IgA in saliva affords protection against bacteria and viruses. There is great interest in the scientific community to determine whether increased levels of respiratory infections in athletes is related to IgA levels in saliva. The few studies that have already been completed show conflicting data. The effects of exercise on IgA levels are still under intense study.

Competitive ELISA:

ELISA- Enzyme-linked immunosorbent assay. ELISA is a technique that is used to determine the level of an antigen (like IgA) in a sample (like saliva). An unknown amount of antigen is affixed to a surface, and then an antibody that can only bind the antigen is added. Once the antibody binds the antigen, excess antibody that is not bound to the antigen is washed off. The antibody is linked to an enzyme. In the final step, a substance is added that the enzyme can convert to a detectable signal. The amount of signal generated is proportional to the amount of antigen initially present in the sample. There are several forms of ELISA but they all follow this general process. In your second lab, you will use the competitive ELISA technique to determine the level of IgA in saliva.

Competitive ELISA is a modification of the general ELISA process. This method uses two antibodies:- the primary and the secondary antibody. The primary antibody is not linked to an enzyme. The primary antibody is incubated with its antigen (IgA) to form an antibody/antigen complex. This complex is then added to an IgA-coated well. The plate is then washed to remove unbound antibody. The rationale is that the more IgA in the sample, the less antibody will be able to bind to the IgA in the well, hence "competition". The secondary antibody, specific to the primary antibody is added. This second antibody is linked to an enzyme. The substrate is added, and remaining enzyme elicits a fluorescent signal. The higher the original antigen concentration, the weaker the eventual signal. The standard curve for the IgA lab will therefore have a shape opposite that of the simple dilutions lab.

Classroom Activity Student Instructions

Role of saliva and salivary IgA in protecting against pathogens:

1. You will be carrying out this activity in groups of 8 students. Each student will take up a role as one of the following:
   - Bacteria
   - Virus
   - IgA secretory receptor
   - First IgA- two students
   - Second IgA- two students
   - Enzyme

   Your group will either be A or B. Group A will not have salivary IgA while group B will not have virus or bacteria.
2. Tape the relevant index card showing your role on your shirt.
3. To start the activity, group B students representing Salivary IgA, enzyme and receptor stand outside the classroom door.
4. Three students: one representing receptor and two representing first IgA hold hands. The second IgA and receptor group similarly hold hands. All

10. At the end of the 10 questions, six or more stuck cards represent an infection if more than half of them are bacteria or epithelial cell death if more than half of the stuck cards are viruses.
11. Group B will now become group A and group A will become group B. The game is then repeated with the same procedure.
12. At the end of the activity, sit in your group. The teacher will provide you with a worksheet that you will discuss and respond to as a group. Write down the responses you agree to on a piece of paper and hand this into the teacher.

Homework:

On construction paper, develop a concept map to describe IgA from synthesis by plasma cells to protection of mucous membrane in the respiratory system.

Secretory IgA Classroom Activity

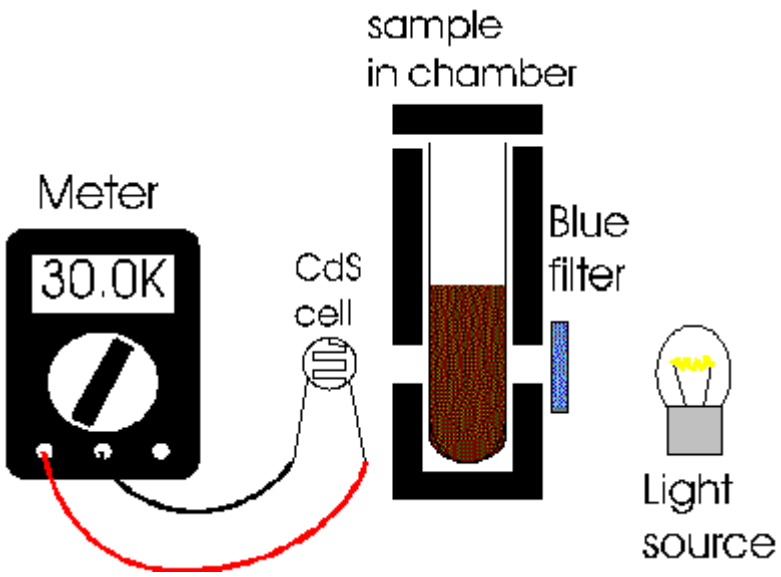
Name
Date

1. Describe the role of each of the following in the process of protection against oral pathogens:
   I.Plasma cells

Simple Dilutions Lab

Spectrophotometer Assembly

The teacher will demonstrate the assembly procedure in front of the classroom.
To assemble the spectrophotometer, follow the diagram below.



Adapted from www.stanford.edu/groups/Urchin/simple.htm

Directions:

4. You have been provided with labeled samples of soft drink (with and without ice) from different sources as your unknowns.
5. Place 10 ml of each sample in a test tube. Label the test tube with the source of the soft drink.
6. Place each standard or sample in turn in the spectrophotometer sample chamber and take the spectrophotometer readings. Record the readings in the table.

Homework:

Complete a lab report. Your report is divided into the following sections:

1. Objectives
2. Materials
3. Methods
4. Results
5. Discussion
6. Conclusion

Your report should be neat, well organized and include all data tables and graphs. It should also include answers to the discussion questions.

Simple Dilution lab
Name
Date

1. What challenges did you encounter during the spectrophotometer assembly?

2. Plot the K-Ohms or absorbance readings (y-axis) from the standards against % soft drink (x axis) on a graph paper or by using a graphing calculator.

3. Determine the concentrations of the labeled unknowns that were collected from stores, restaurants and movie theaters by reading their K-Ohms or absorbance value from the spectrophotometer, and determining the corresponding % soft drink value on the from the standard curve. Record your data in the table below.

Sample source	K-Ohms or absorbance	% soft drink
Store 1		
Store 2		
Store 3		
Rest. 1		
Rest. 2		
Rest. 3		
Movie theatre 1		
Movie theatre 2		
Movie theatre 3		

4. Compare your data with data from other groups. Is the data consistent? If not, what could be the source of the differences?

5. What sample had the highest soft drink concentration without ice?

6. What sample had the highest concentration of soft drink with ice?

7. Suggest possible reasons why the soft drink concentrations were different between the samples.

8. Assuming the stores, movie theatres and restaurants are all located in one mall, where would you purchase soft drink and why?

9. In this lab, you did a linear dilution. How would you do a serial dilution based on a factor of 10? (starting from the same 100% dilution)

10. In the next lab, you will use the dilution technique to dilute secretory IgA. What is the relationship between K-Ohms readings and concentration from your graph?

11. How would you record the results for a solution that changes color with time?

12. What would happen if the samples were not mixed well? How would this affect the readings?

Secretory IgA Lab

sIgA ELISA Instructions:

1. You will work in pairs in this laboratory exercise.
2. Wear goggles, lab apron and gloves during the entire lab. The teacher will demonstrate the correct way to remove gloves.
3. Rinse mouth with water 10 minutes before saliva collection.
4. In the first table of the lab procedure provided by the teacher, record your last name initials (one initial from each student) as the sample ID.
5. Place one end of the 5cm straw piece in your mouth and the other end in the microcentrifuge or whatever tube your teacher provides.
6. Imagine taking your favorite food (yummy!) and allow saliva to pool in the mouth.
7. With head tilted forward, drool (this is fun!) down the straw and collect saliva in the microcentrifuge. Note- it is normal for saliva to foam.
8. Repeat until your saliva (minus foam) hits the 1mL mark.
9. Your partner will record the time it takes to collect 1mL.
10. If your mouth is dry, gently chew on the end of the straw to stimulate saliva production.
11. Label the tubes with the same initials you used for the sample ID.
12. Using a 1000ul pipette, transfer the 1mL saliva into your partners microcentrifuge tube. Use the pipette to thoroughly mix the saliva. Remove 1mL of this mixture and place it back in the original tube so you have 1mL of the mixture in both tubes.
13. Snap the tubes shut and gi

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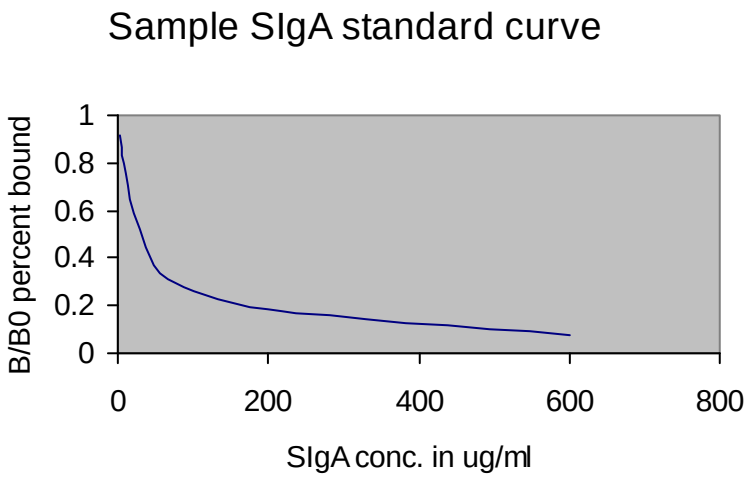
3. The teacher will provide the optical density readings for all samples and standards. Copy the table provided by the teacher.

4. Calculate the percent bound (B/Bo) for each standard, control and unknown by dividing the average OD (B) by the average OD for the zero (Bo)

Well	Sample	Average OD	B	B/Bo	SIgA (ug/ml)

5. On graphing paper, graph percent bound B/Bo (y-axis) versus SIgA concentration in ug/ml (sIgA conc. In ug/mL). Below is a sample graph (your graph may not look exactly like this).



6. Determine the concentrations of the controls and unknowns by interpolating from the standard curve. The unknowns will be the mixed student samples and will be labeled with student initials. Record the data in the table below.

7.

Sample	Concentration (g/ml)	X5 to obtain absolute SIgA conc.
1-Low control		
2-High control		
Unknown1		
Unknown 2		
Unknown3		
Unknown 4		
Unknown 5		
Unknown 6		
Unknown 7		
Unknown 8		
Unknown9		
Unknown 10		
Unknown 11		
Unknown 12		
Unknown 13		
Unknown 14		
Unknown 15		
Unknown 16		

8. Multiply the concentrations of unknown saliva samples by 5 to obtain the final concentration of SIgA in ug/ml.

9. Calculate corrected sIgA concentration.

Corrected SIgA= absolute SIgA (ug/mL) x flowrate (mL/min)

Sample ID	Absolute SIgA (ug/mL)	Flow rate (mL/min)	Corrected SIgA (ug/min)

10. What is your group's corrected sIgA concentration? Is it different from the absolute concentration obtained in step 6 above?

11. What factor led to the difference between the two sIgA concentrations?

12. Describe the difference between the standard curve from the simple dilution experiment and the standard curve from salivary IgA ELISA.

13. Are there significant differences between the groups in IgA concentration?