

# The Mysterious Meat Allergy

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Additional information regarding the CATALySES project is available at <https://www.cpet.ufl.edu/teachers/catalyses/>

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## Author's Note

I am a marine ecologist by training, so designing a curriculum sequence focused on an allergic immune response to a carbohydrate associated with red meat that is connected to the bite of a terrestrial tick species could not be further from my area of expertise! But I love the way that the many different fields of science are interconnected and can lead you into conversations with people that are in entirely different disciplines. As a teacher I love to seed the classroom with bread crumbs in the form of case studies, real world data, and hands on opportunities that will generate genuine inquiry and discussion as students follow the path with their peers to arrive at a reasonable and well supported conclusion.

This curriculum came together over an extended period of time. The inspiration for the “hook” in this unit came from my love of podcasts. The producers of Radio lab tell a riveting story of the emergence of the Alpha Gal Red Meat allergy (the link to which is available throughout this curriculum) the same way they do with all of their science based stories by weaving scientific concepts and discoveries into genuine storytelling. As teachers we rarely create or implement on our own, Elizabeth Emery from Leesburg High School worked to create the foundational component of this lesson relating Alpha Gal to the abstract concept of macromolecules that our students often struggle with, I simply added to it.

The opportunity to work with the Emerging Pathogens Institute and the lab of Dr. Gregory Glass opened my eyes up to the world of medical geography. Learning a new skill like geographic information systems or how to interpret the data used to build their related models is valuable extension of the abiotic/biotic interactions we often preach to our students. By providing some insight as to how those GIS models are built, students can see opportunities for careers that may parallel a more traditional science career. And never has been building and improving on these models been more important. As climate change shifts many of the variables that influence aspects of the biological sciences like species distribution and emerging pathogens it is essential to continue the work in acquiring accurate data to refine those models in hopes of predicting future outbreaks or changes in the complex interactions that exist within the world's ecosystems. See it comes down to ecology after all!

Whilst the transmission of the Alpha Gal meat allergy exists at the species to species interaction level, and the modeling to identify the likely location of the organisms that transmit it exist at an ecosystem level, the utilization of molecular techniques should not be ignored. By understanding how the immune system interacts with pathogens associated with host organisms like ticks we can better design drugs to protect against them. Using biotechnology to further refine our understanding of immune responses as well as extract DNA associated with these pathogens for identification and comparison can also tell how these pathogens are related, how they evolved and what ways we can continue to use emerging biotechnology applications to reduce their impact to human health.

## Introduction

Getting students to understand complex concepts in science requires us to make them care about why they should learn it. I can think of few other ways to speak to a high school student than to focus on what they eat and introduce the possibility that what they ate one day without worry could create a significant allergic reaction the next time they eat it! The Galactose-1,3-Alpha-Galactose (Alpha Gal) red meat allergy is an intriguing case in several ways. The allergy did not gain significant attention from medical researchers or scientists until only recently. Tracing the cause of the Alpha Gal allergy to the Lone Star Tick (*Amblyomma americanum*) was a collaborative effort across fields of science and other disciplines and involved the work of researchers working on a clinical trial for a drug designed to treat cancer, immunologists to understand the molecular pathways associated with the allergic reactions, medical geographers and entomologists that specialize in the distribution of ticks around the country! Tick species are also associated with a variety of other pathogens and diseases. In fact it was the overlap of certain tick borne pathogens and their disease outbreaks and the occurrences of red meat allergies that allowed the researchers to connect the dots and identify the Lonestar tick as the likely culprit. The distribution of tick species are on the move as they no doubt respond to changes in environmental factors that influence their life cycle such as climate conditions, host distribution, and habitat availability. The emergence of the red meat allergy brings out the fundamentals of science: Using curiosity to ask good questions, making ongoing observations, analysis of data and collaboration with colleagues to generate conclusions. There is no reason that the students in our classroom cannot accomplish a similar feat.

## 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.

**STUDENT PAGES:** Worksheets and handouts to be copied and distributed to the students.

**TEACHER MASTERS:** Versions of the student pages with answers or the activity materials for preparation.

**Collaborative Learning:** The lessons in this curriculum have been developed to include many collaborative learning opportunities. Rather than presenting information in teacher-driven, lecture format, the activities involve the students in a more engaged manner. For classrooms not accustomed to using collaborative learning strategies, have patience. It can be difficult to communicate instructions, particularly for students who are visual learners. For these students, use of visual clues such as flowcharts and graphics can help them understand how they are to move to different groups.

**Groups:** Most of the lessons are carried out in groups. While it isn't necessary for students to remain in the same groups the entire unit, if they work well together, it may foster students to think deeper as they are comfortable with their teammates and willing to ask questions of each other.

**Inquiry-based:** The lessons in the curriculum invite students to be engaged and ask questions. They work through background information in a guided fashion, but are challenged to think beyond what they have read or done. The teacher serves as the facilitator in these activities, not the deliverer of information.

**Technology:** Lessons have been written to be mindful of varying availability of technology in schools and homes. Some of the lessons would be very well suited to online environments and if your students are able, you might wish to engage in some of the technology modifications.

**Content:** This unit provides an opportunity to synthesize discrete content facts into an authentic context. Students take concepts learned such as immune response and clinical testing procedures, and put them in the context of emerging pathogens. Although effort has been made to teach the fundamentals of the immune system, this is but an overview of one facet of the immune response, rather this curriculum is designed to show students how different disciplines are interconnected in the realm of human health. The curriculum also assumes that students have a basic understanding of biochemistry (macromolecules) and are familiar with different forms of biotechnology.

**Implementation notes:** This curriculum should be modified and adapted to suit the needs of the teacher and students. To help make implementation easier in this first draft, notes have been included in lessons as needed.

**Extensions:** There are many opportunities to expand the lessons presented here. When appropriate extensions are provided with supporting materials. Elsewhere in the curriculum suggestions as to how to personalize or extend the content for your students are provided.

**Science Subject:** Advanced Placement Biology

**Grade and ability level:** 9-12 students in Advanced Placement Biology, can be modified for 9th grade Biology/Honors Biology

**Science concepts:** Macromolecule structure/function, immune response, emerging pathogens, GIS modeling and Biotechnology techniques

## **Lesson Summaries**

### **Lesson One: Meat No More Case Study and Background Jigsaw**

Students will use a fictional case study inspired by a variety of real world incidences of alpha gal reactions to acquaint themselves with the symptoms and role of epidemiologists in diagnosing a patient. In part 2 the students review basic macromolecule function, biotechnology techniques and aspects of the immune response associated with the alpha gal allergy.

### **Lesson Two: Steps of an ELISA and Application of the ELISA procedure to identify Alpha Gal Antibodies**

Student match diagrams with text descriptions to understand the steps of an ELISA. A common test used to detect if a patient has been exposed to a specific antigen is called an ELISA (enzyme linked immunosorbant assay). This test takes advantage of the interactions between antigens and antibodies. Often compared to a lock and key, an antigen/antibody interaction is very specific. Part 2 of this lesson allows them to put their understanding into practice as they using a commercial, classroom-friendly ELISA kit, students will test the patient serum sample for the presence of dengue antibodies, and record their results on the epidemiological report. A simulated version is also presented.

### **Lesson Three: GIS Modelling**

Students will map the location of other occurrences of the alpha-gal red meat allergy and assume the role of a medical geographer as they match the distribution of the allergy cases with that of the different ticks species common to the United States. Once identified, students will use GIS Modelling data to identify relationships between the Lone Star ticks distribution and environmental factors that might influence its survival and ability to transmit the alpha gal red meat allergy.

### **Lesson Four: DNA Extraction and Optional PCR**

Using a commercially available DNA extraction kit, students will simulate the extraction of DNA from “preserved lone star ticks”. If both PCR equipment and Gel electrophoresis equipment is available in the classroom lab, a simulated PCR can be conducted using a different commercially available kit.

### **Lesson Five: Optional Electrophoresis or BLAST Procedure**

If the PCR in lesson four was conducted, another commercially available kit can be used to simulate the process of an electrophoresis where students will attempt to identify potential pathogens that the tick species endemic to the southeastern region of the United States carry. If the PCR was not run gene sequences can be analyzed using the Basic Linear Alignment Sequence Test (BLAST) available through the National Institute of Health to identify the vector based DNA present in the extracted DNA samples.

### **Lesson Six: Optional Journal Article Review:**

Using the article “The alpha-gal story:Lessons learned from connecting the dots students can engage in a class wide discussion of the process of identifying the relationships between existing



clinical trials, immunology, and the distribution of the lone star tick in an effort to bring the unit to a close.

## Lesson Sequencing Guide

Since the classroom teacher knows his or her students best, the sequencing of lessons and the amount of time spent on each should be altered to meet the needs of each individual setting. Below is a suggested pacing guide that can be used when planning to use this curriculum, assuming 84-minute block class periods, if you teach in a more traditional 45 minute class schedule the activities have been set up so that they can be taught across two days, which would double the expected delivery time.

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
<p><b>Lesson 1</b> Part 1 <b>Meat no more?</b> (Case Study Introduction) <b>20 minutes</b></p> <p>Part 2 <b>Background Information</b> (Jigsaw Activity) <b>60 minutes</b></p> <p>Optional Extension <b>Pathogens, Antibodies and Vaccines- Part 1</b> (Science Takeout Activity)</p>	<p><b>Lesson 2</b> Part 1 <b>Steps of an ELISA</b> (Sorting Activity) <b>(35 minutes)</b></p> <p>Part 2 <b>Testing for Alpha Gal Antibodies</b> (Hands on simulation) <b>(45 minutes)</b></p>	<p><b>Lesson 3</b> Part 1 <b>Looking for Patterns</b> (Alpha Gal/Tick Disease Mapping/Matching Activity) <b>(30 minutes)</b></p> <p>Part 2 <b>GIS Modelling</b> (Computer Simulation) <b>(50 minutes)</b></p> <p>Optional Extension <b>Ghost Moose Article</b></p>	<p><b>Lesson 4</b> Part1 <b>The Alpha Gal Story:Lesson Learned from Connecting the Dots Article Discussion</b> <b>(45 minutes)</b></p>	<p><b>Lesson 5</b> Part 1 <b>Tick Pathogen DNA Simulated Extraction</b> <b>Optional PCR (80 minutes and overnight Incubation)</b></p>	<p><b>Lesson 6</b> Part 1 <b>Tick Pathogen Electrophoresis and Identification</b> (60 minutes) Part 2 <b>Or Pathogen Gene Sequence BLAST Activity</b> <b>(40 minutes)</b></p>

<sup>A</sup>: The length of this lesson can vary based on the prior knowledge of the students. Some can be assigned as homework to conserve in-class time.

## Vocabulary

## Next Generation Sunshine State Standards - Science

AP Biology Curriculum Standards	Lesson					
	1	2	3	4	5	6
<b>Essential knowledge 2.D.4: Plants and animals have a variety of chemical defenses against infections that affect dynamic homeostasis</b> <b>(B)</b> Mammals use specific immune responses triggered by natural or artificial agents that disrupt dynamic homeostasis.	X	X			X	X
<b>Essential knowledge 3.A.1: DNA, and in some cases RNA, is the primary source of heritable information</b> <b>(E):</b> Genetic engineering techniques can manipulate the heritable information of DNA and, in special cases, RNA.				X	X	
<b>Essential knowledge 4.A.1: The subcomponents of biological molecules and their sequence determine the properties of that molecule</b>	X					X
<b>Essential knowledge 2.D.1: All biological systems from cells and organisms to populations, communities and ecosystems are affected by complex biotic and abiotic interactions involving exchange of matter and free energy.</b> <b>(C):</b> The stability of populations, communities and ecosystems is affected by interactions with biotic and abiotic factors. [See also 4.A.5, 4.A.6]			X	X		
<b>Science Practice 1:</b> The student can use representations and models to communicate scientific phenomena and solve scientific problems			X			
<b>Science Practice 4:</b> The student can plan and implement data collection strategies appropriate to a particular scientific question		X	X	X	X	
<b>Science Practice 5:</b> The student can perform data analysis and evaluation of evidence	X	X	X	X	X	X
<b>Biology Next Generation Sunshine State Standards</b>						
<b>SC.912.L.14.52</b> Explain the basic functions of the human immune system, including specific and nonspecific immune response, vaccines, and antibiotics.	X	X			X	X
<b>SC.912.L.14.6</b> Explain the significance of genetic factors, environmental factors, and pathogenic agents to health from the perspectives of both individual and public health.	X	X	X	X	X	X
<b>SC.912.L.18.1</b> Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules.	X			X	X	
<b>SC.912.L.16.10</b> Evaluate the impact of biotechnology on the individual, society, and the environment, including medical and ethical issues.		X		X	X	
<b>SC.912.L.16.11</b> Discuss the technologies associated with forensic medicine and DNA identification, including restriction fragment length polymorphism (RFLP) analysis.		X		X	X	
<b>SC.912.L.16.12</b> -Describe how basic DNA technology (restriction digestion by endonucleases, gel electrophoresis, polymerase chain reaction, ligation, and transformation) is used to construct recombinant DNA molecules (DNA cloning).					X	
<b>SC.912.N.1.1</b> Define a problem based on a specific body of knowledge, for example: biology, chemistry, physics, and Earth/space science, and do the following: a. pose questions about the natural world; b. conduct systematic observations; c. examine books and other sources of information to see what is already known; d. review what is known in light of empirical evidence;	X	X	X	X	X	

<ul style="list-style-type: none"> <li>e. plan investigations;</li> <li>f. 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);</li> <li>g. pose answers, explanations, or descriptions of events;</li> <li>h. generate explanations that explicate or describe natural phenomena (inferences);</li> <li>i. use appropriate evidence and reasoning to justify these explanations to others;</li> </ul>					
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## Background information:

### The Case of the Mysterious Meat Allergy Teacher Background

The background information below provides an overview of the aspects important to understanding the structure of the galactose-alpha-1,3-galactose (alpha-gal) carbohydrate, its role in generating an immune response and the subsequent molecules associated with that immune response (structure and function) as well as the methods used by medical geographers to better identify locations where ticks live and tick borne diseases are located. More detailed information can be obtained from the scientific literature and other relevant sources provided at the end of this section.

### What is Galactose-alpha-1,3-galactose (alpha gal)?

As the suffix implies, Galactose-alpha-1,3-galactose (alpha gal) is classified as a carbohydrate or sugar molecule. Specifically alpha gal is an oligosaccharide, meaning it is made up of a small number of repeating carbohydrate monomers. The alpha gal carbohydrate is present in the tissues of all non-primate animals excluding animals that would not be considered white meat such as chicken or fish, which is why it is associated as a “red meat allergy that can result in hives, fever, intestinal distress and in more severe cases anaphylactic shock.

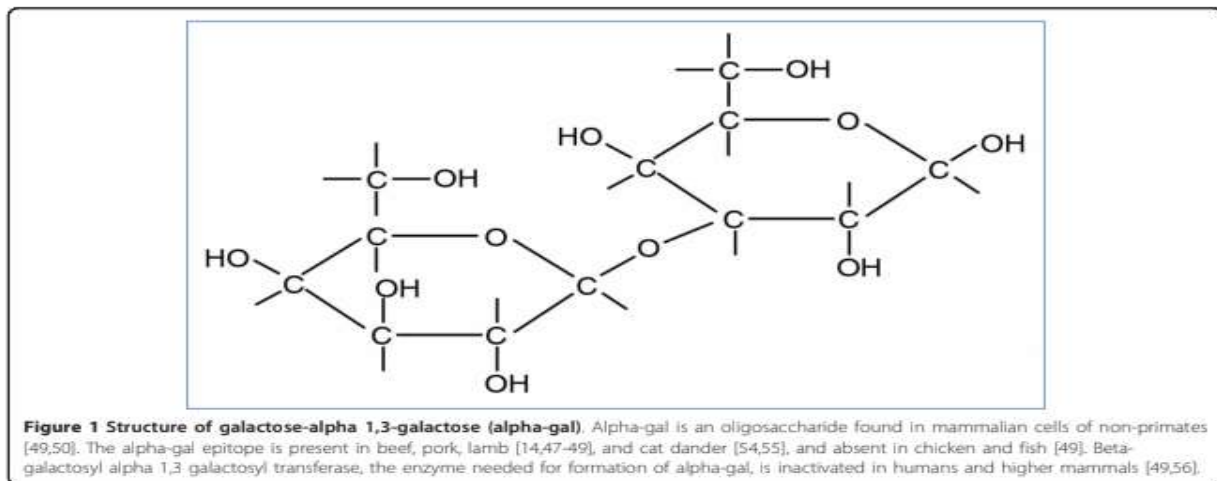


Image from (Saleh et al. 2012)

The structure of the alpha carbohydrate is such that a portion of the molecule (known as an epitope in regards to a human immune response) can, in certain people, be recognized as an antigen by the human immune system, and thus result in an immune response. Humans and more complex mammals contain the gene necessary to generate an enzyme (Beta-galactosyl alpha 1,3 galactosyl transferase) that assists in the formation of alpha gal, however, that gene is inactivated.

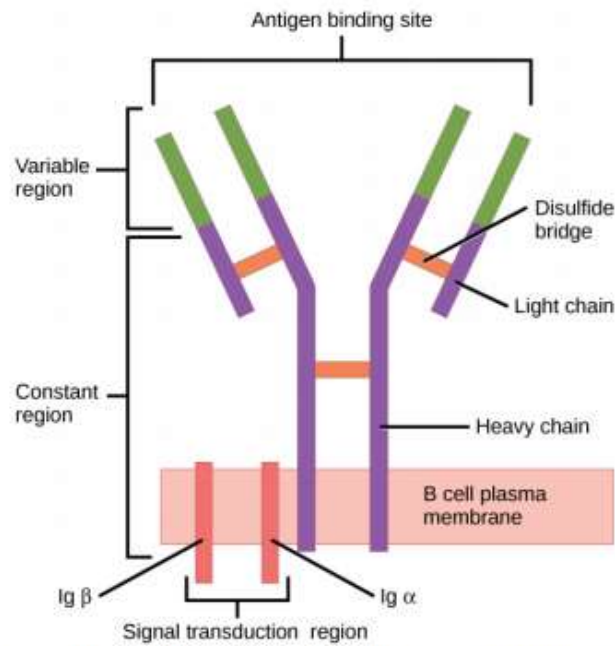
Not all tissues associated with red meat have the same concentrations of alpha gal, investigations related to meat allergies have identified certain organs may have higher concentrations of alpha gal. Specifically the kidney which plays a role in metabolizing alpha gal may have high concentrations of the molecule and thus eating kidney, a habitat which is more common in European countries, can produce more pronounced allergic reactions.

## **Immune Response**

The immune response is an evolutionary characteristics that all animals share from the simplest organisms in the Phylum Porifera (sponges) to complex mammals. The degrees of complexity associated with the immune response are related to the complexity of the organism itself. Thus humans have evolved a complex system to protect internal homeostasis from viruses, viral particles, bacteria or other potential “invaders”. The immune system is made up of two distinct stages, the innate immune system which is always present and defends against all pathogens, and the adaptive immune system stores “information” about previous infections and and illicit specific immune responses to future infections of the same pathogen.

Extracellular “invaders” are identified by what are known as pathogen-associated molecular patterns which are essentially an “invaders” calling card and may either be carbohydrates, peptides or nucleic acids. The immune system identifies these molecules as “nonself” molecule and will use the many different molecules and cells of the immune system to recognize and destroy the “non-self invader”.

Players in the immune response to alpha-gal which generates the “red meat allergy” are antigen presenting cells, T-Helper cells (specifically T-helper-2 cells) which in turn stimulate unspecified B cells which will in turn secrete specific antibodies. It is the B cells that will generate the direct immune response that can result in some of the symptoms associated with alpha-gal (red meat allergy). B cells have two sets of side chains, one of which is directly responsible for binding antigens.



**Figure 33.13** B cell receptors are embedded in the membranes of B cells and bind a variety of antigens through their variable regions. The signal transduction region transfers the signal into the cell.

Image Zedalis, J., Eggebrecht, J. (2018).

As mentioned earlier in regards to the alpha-gal molecule, a particular portion of that molecule (the epitope) will fit, much like a lock and key, with the antigen binding site. The resulting antigen/epitope B cell interaction activates cells called mast cells which in turn generate molecules like histamine that can lead to specific symptoms associated with the like hives, fever and anaphylaxis.

histamine that can symptoms “red-meat allergy” and swelling and

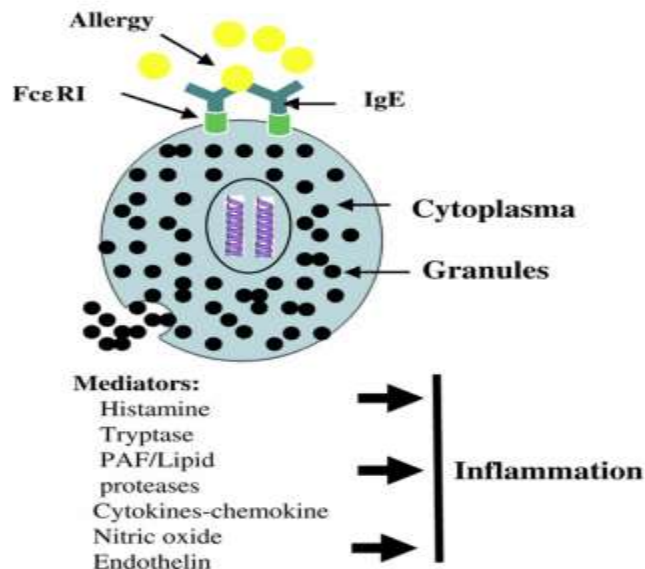


Image from Amin (2011)

**Figure 1** The IgE-primed mast cell releases granules and powerful chemical mediators, such as histamine, cytokines, granulocyte macrophage colony-stimulating factor (GM-CSF), leukotrienes, heparin, and many proteases into the environment. These chemical mediators cause the characteristic symptoms of allergy.

sterious Meat Allergy



## Relationship Between Alpha-Gal and Tick Bites (*Lone Star Tick-Amblyomma americanum*)

Typically medical treatment related to tick bites is sought after the development of symptoms associated with a variety of viral or bacterial vectors that tick species are associated with such as Rocky Mountain Spotted Fever which is associated with the *Rickettsia rickettsii* bacteria and is often delivered via the Dog Tick (*Dermacentor variabilis*), Lyme disease (*Borrelia burgdorferi* bacteria and the Black Legged Tick (*Ixodes scapularis*). The tick life stage starts with the larval form which are typically called seed ticks as they are picked up from their habitat in high numbers and are very small in size. In order to progress to the next life stage (nymphs) a blood meal must be taken. Typically this meal will be taken from small rodents that have overlapping distribution with the tick species. Some tick diseases can be inherited from the previous generation, but many of the bacterial vectors that are transmitted by ticks are picked up at the nymph stage from the first blood meal. In order to locate a blood meal, a tick will “quest” where it climbs too high points of the ground cover and uses the many hook like projections to grab hold of the future host as it passes by. Some species of ticks are sensitive to carbon dioxide and can use this sensitivity to alert themselves to a nearby host. Both male and female ticks require blood meals, however, the female in particular needs a blood meal in order to produce eggs that will give rise to the next generation of larval ticks.



**Figure 3.** Life stages of lone star ticks, *Amblyomma americanum* (Linnaeus), from top left clockwise: larva, nymph, adult male, adult female. Photograph by [Chris Holderman](#).



**Figure 9.** Blood-fed, engorged female lone star tick, *Amblyomma americanum* (Linnaeus). Photograph by Lyle Buss.



**Figure 4.** Female lone star tick, *Amblyomma americanum* (Linnaeus), with egg mass. Photograph by Lyle Buss.

Holderfield and Kaufman (2017)

[http://entnemdept.ufl.edu/creatures/urban/medical/lone\\_star\\_tick.htm](http://entnemdept.ufl.edu/creatures/urban/medical/lone_star_tick.htm)

The relationship between the alpha-gal carbohydrate/red meat allergy and bite from the Lone Star tick is an intriguing opportunity for students to explore the scientific process. The realization that there may be a correlation between tick bites and this allergy was associated with clinical trials related to a drug designed to treat colorectal cancer. The drug (Cetuximab) produced outlier observations related to increased allergic responses and those responses had several things in common: People that presented with this allergy to the drug were predominantly in the southeastern portion of the United States, and many of them had a lifestyle that took them outdoors often to environments that are ideal tick habitat. Many of the the people presenting with an increased allergic reaction often reported receiving recent tick bites.

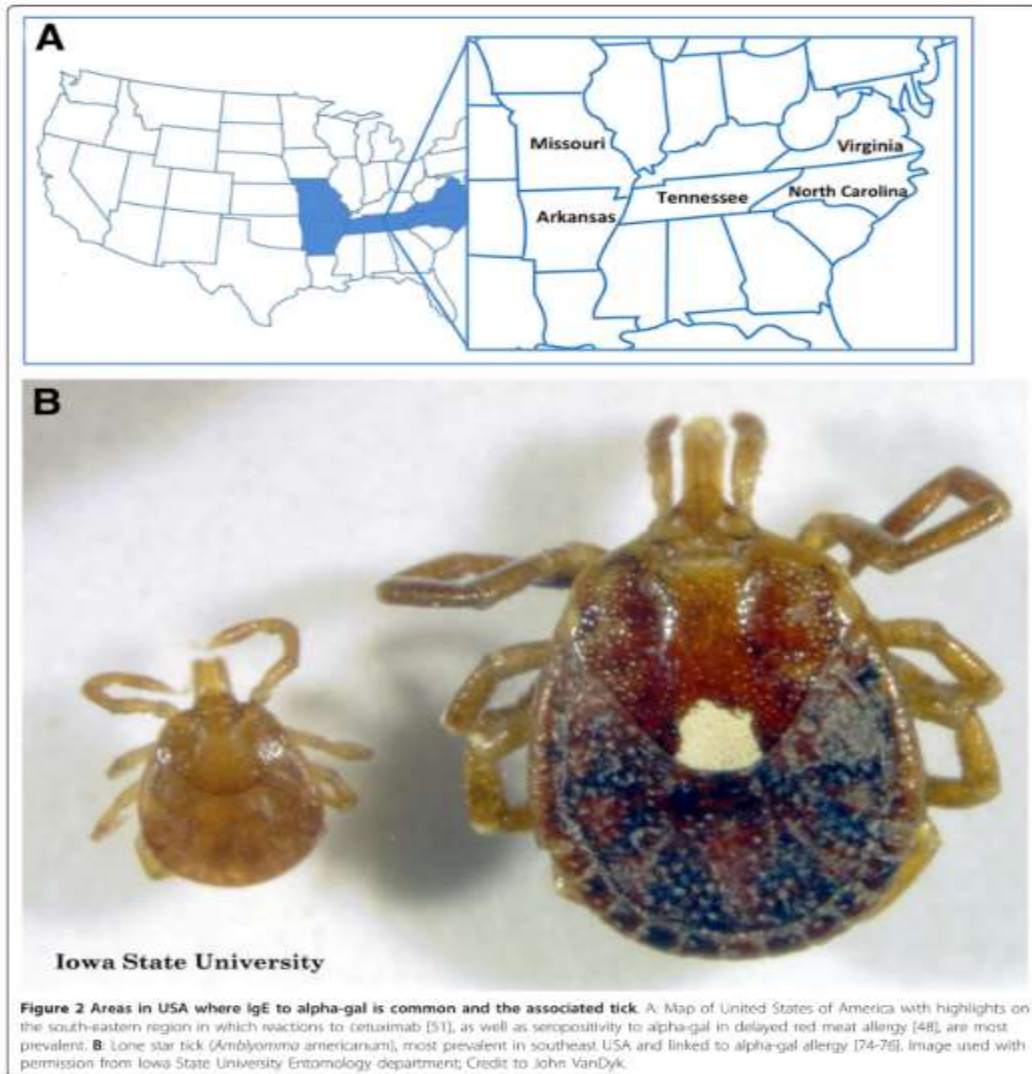


Image from Saleh et al. (2012).

A specific component of the drug Cetuximab had a region that was similar in molecular structure to the epitope associated with the alpha-gal allergy. So in effect, the reason that the people were demonstrating a heightened sensitivity to the drug was that something associated with the tick bite had increased their immune response to this particular structural component of the drug.

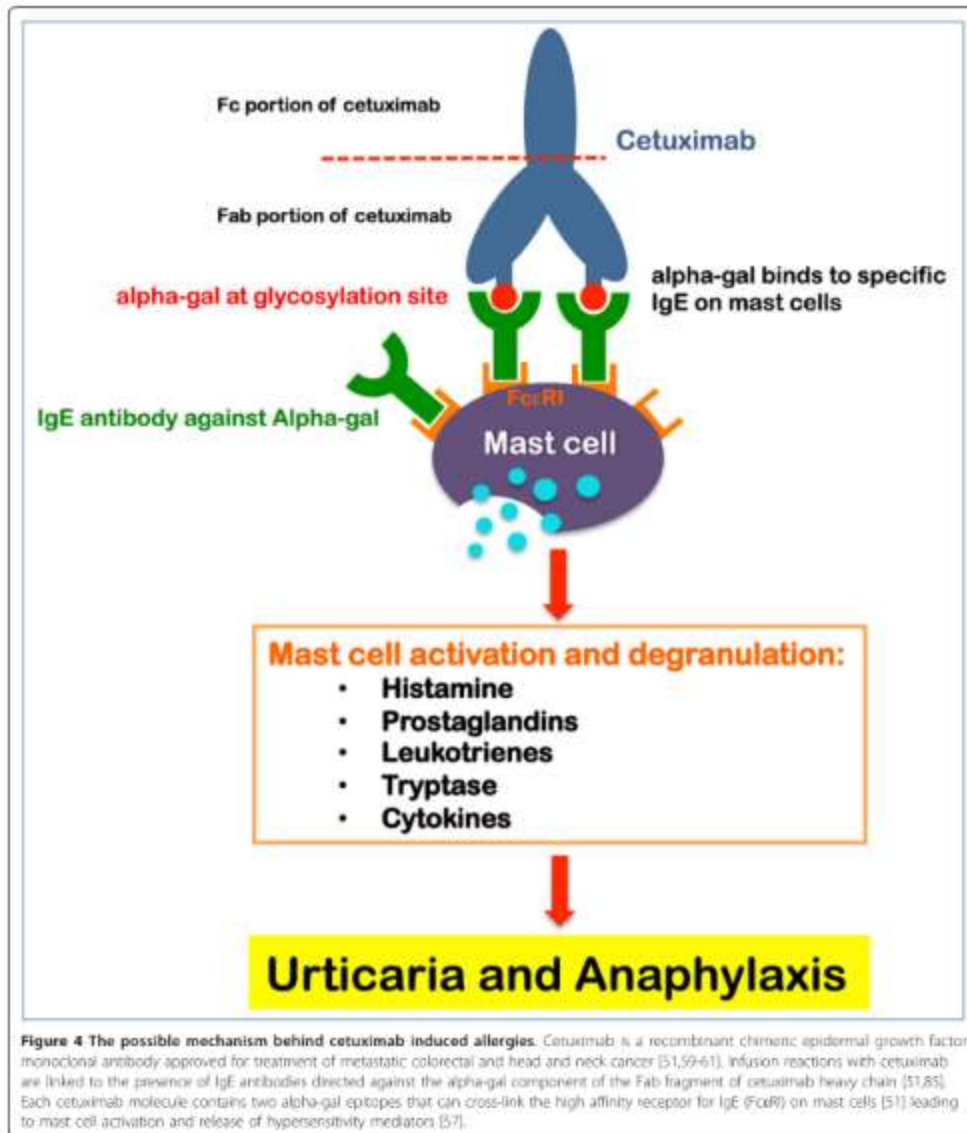
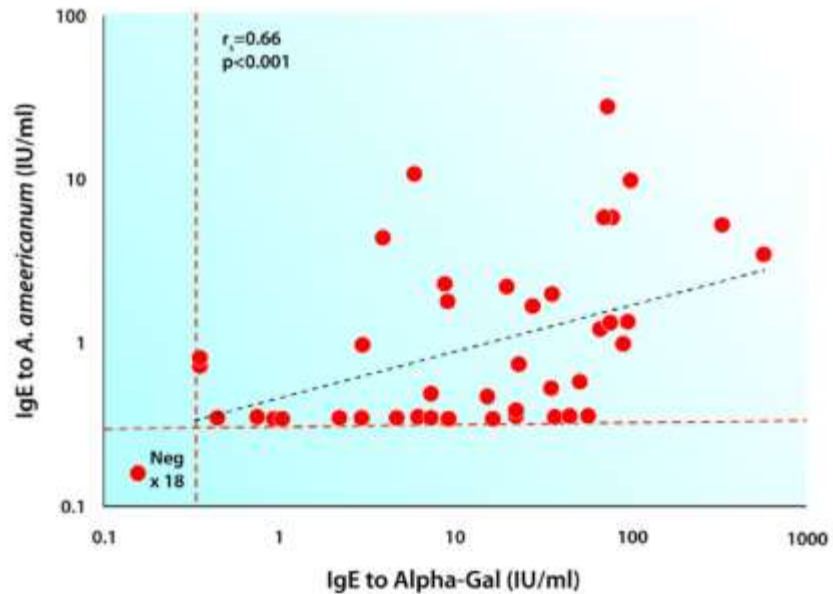


Image  
Saleh et al. (2012)

from

The figure here illustrates a significant correlation between red meat allergy presenting patients' IgE response to the Lone Star tick and the same patients' IgE response to the alpha-gal carbohydrate. The alpha-gal meat allergy is not isolated to the southeastern United States. Cases have been identified in places like Sweden and Australia. Researchers in the other places where the allergy has popped up are confident that ticks are the predominant cause of this heightened sensitivity to meat, although in Europe and Australia the tick species are different (*Ixodes ricinus* and *Ixodes holocyclus* respectively)



**FIG 2.** Relationship of IgE to alpha-gal with IgE to *Amblyomma americanum* (lone star tick). Levels of specific IgE to alpha-gal and *A americanum* were measured by using ImmunoCAP, and the correlation between the 2 types of IgE was determined by using Spearman correlation ( $r_s = 0.66$ ,  $P < .001$ ).

mage  
from  
Steinke et al. (2015).

## Geographic Information Systems (GIS) Models

GIS maps are an increasingly important part of the biological sciences. While we may think of using GPS to locate our position or help us get to our next destination, GIS maps can take GPS coordinates and superimpose them with important ecological abiotic and biotic factors. Of particular importance to this curriculum is the distribution of different tick species throughout the United States. As described in the [Radiolab](#) podcast that was the inspiration for this curriculum it was the overlap of presented cases of the alpha-gal meat allergy and the occurrences of specific tick borne diseases that helped identify the relationship and eventual vector that is contributing to this allergy.



## Geographic Distribution of Nationally Notifiable Tickborne Diseases, 2015

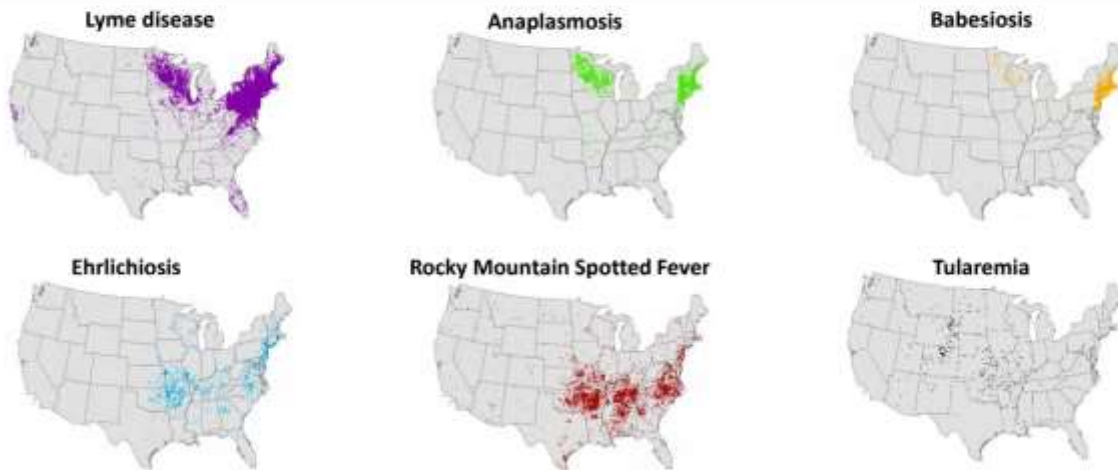


Image from Eisen (2012)

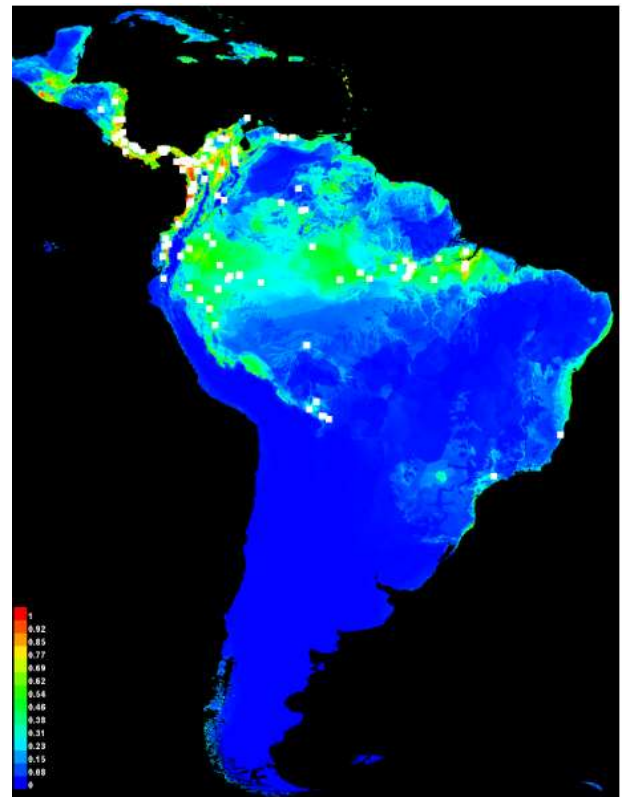
Identifying the occurrences of tick borne diseases themselves can be incorporated into GIS maps by looking at patient data and where the cases were presented. What medical geographers using GIS programs aim to do is to refine the maps of the species that are the major vectors for the diseases which means locating areas where ticks live and then using biotechnology techniques (DNA extraction, Polymerase Chain Reactions (PCR), Gel electrophoresis and genetic sequencing) to identify if those populations of ticks are associated with the viral and bacterial pathogens associated with the diseases.

The small size of ticks make them easy to miss in habitats that they may be readily abundant with (unless you happen to have one taking a blood meal from you), and one would think that techniques to identify tick population hotspots would require complex techniques. Although the eventual GIS maps generated require complex coding in statistical programs and specialized GIS software, locating ticks is actually relatively simple. The process is called flagging. This technique requires a simple GPS device such as a smartphone or tablet, and a piece of cloth attached to a broom handle. A research will walk a straight line transect of predetermined length sweeping the cloth back and forth. The anatomy of the tick lends itself to being swept up on the “flag” where it is removed and preserved for future identification by the field researchers.



Image courtesy of Dr. Glass (University of Florida) Left-A Lone Star tick (*Amblyomma americanum*) on a flag. Right- Field researchers with tick flags in hand before a transect.

GPS coordinates of ticks located in the field can be correlated to environmental conditions obtained from a variety of databases such as the United States Geological Survey or [worldclim.org](http://worldclim.org). The importance of using GIS programs is it can identify areas where specific species of ticks are found, identify the biotic and abiotic conditions of those sites and use it to generate a model that can predict tick distribution through a wider geographic region. GIS programs in combination with statistical applications can identify the biotic or abiotic factors that have the greatest influence on the presence, absence, population densities etc. of the species of interest. This is of particular importance as many abiotic and biotic factors will be disrupted as global climate change continues to alter local environmental conditions.



## Relevant Literature

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## Lesson 1: Meat No More? A Curious Case of the Immune Response

### Lesson 1 Part 1: Narrative and Case Report

**KEY QUESTION(S):** How does the immune system identify and respond to red meat in order to develop an immune response?

**OVERALL TIME ESTIMATE:** Part 1-20 minutes, Part 2-60 minutes

**LEARNING STYLES:** Part 1-Auditory, Part 2-Verbal, Social

#### **VOCABULARY:**

Galactose-1,3-alpha Galactose (Alpha Gal)

Antibodies

IgE

**LESSON SUMMARY:** Students will listen to the scenario establishing the alpha gal (red meat allergy) and will identify the associated symptoms while hypothesizing a potential cause for the recent onset of this allergy. After the case study has been introduced, students will obtain valuable background information regarding the immune response through a collaborative jigsaw activity.

#### **STUDENT LEARNING OBJECTIVES:**

The student will be able to...

- Interpret a case report
- Hypothesize possible factors contributing to an illness
- Obtain additional background information through collaborative note taking

#### **FLORIDA NEXT GENERATION SUNSHINE STATE STANDARDS (SCIENCE):**

##### **AP Biology Curriculum Standards**

**Essential knowledge 2.D.4:** Plants and animals have a variety of chemical defenses against infections that affect dynamic homeostasis- **(B)** Mammals use specific immune responses triggered by natural or artificial agents that disrupt dynamic homeostasis.

**Essential knowledge 4.A.1:** The subcomponents of biological molecules and their sequence determine the properties of that molecule

**Science Practice 5:** The student can perform data analysis and evaluation of evidence

##### **Next Generation Sunshine State Science Standards**

**SC.912.L.14.52** Explain the basic functions of the human immune system, including specific and nonspecific immune response, vaccines, and antibiotics.

**SC.912.L.14.6** Explain the significance of genetic factors, environmental factors, and pathogenic agents to health from the perspectives of both individual and public health.

**SC.912.L.18.1** Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules.

**SC.912.N.1.1** Define a problem based on a specific body of knowledge...

**MATERIALS:**

Part 1-Copies of Case Report for Each Student

Part 1/2-Copies Jigsaw Note Taking Handout for Each Student

Part 2-Cutouts of each of the jigsaw pages for each group

Optional Extension-Science Take-Out Kit [Pathogens, Antibodies, and Vaccines](#) Catalog #STO-138

**BACKGROUND INFORMATION:**

The **Galactose-1,3-Alpha Galactose (Alpha Gal)** allergy first appeared around 2009 and since then has increased in its frequency. Patients presenting with this allergy often do so 3 to 6 hours after consuming red meat. Patients present with **urticaria** (hives), **angioedema** (swelling under the skin), or recurrent **anaphylaxis** (allergic reaction). Alpha Gal is a disaccharide sugar that is present on the surface of tissues of red meat. Humans and other primates lack the enzyme that is an important step in the metabolic pathway that creates this carbohydrate, as a result it is typically absent in the human system unless ingested as part of the patients diet. An immune response is not typical when this carbohydrate is ingested, otherwise, the majority of humans would be unable to eat red meat, but rather is the result of an overactive immune response resulting from some other factor the patient has experienced. The immune response associated with the Alpha Gal allergy follows a typical immune response pathway Antigen presenting cells, **T-Helper** cells (specifically **T-helper-2 cells**) stimulate **unspecified B cells** which will in turn secrete specific **antibodies** called **immunoglobulin E (IgE)**. A particular portion of the IgE molecule will fit with a portion of the antigen called an epitope much like a lock and key, that will activate mast cells which in turn release chemical messenger molecules like histamines that will generate the symptoms related to the allergic response such as hives, rash, swelling etc.

**ADVANCE PREPARATION:**

Copy of case report for each student

Copy of Jigsaw Note Taking Handout for each student

Cut outs of each of the jigsaw pages (1 per group) organized into envelopes/clipped together for easy distribution/collection For extended use, recommend laminating jigsaw cards.

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

Part 1-20 minutes

1. Distribute a copy of the Case Report per student.
2. (5 minutes) Tell the students this is the actual case report as recorded by the CDC. Ask students to read the case report silently or narrate the story to the class as they follow along.
3. Tell the students they are now an epidemiologist on the case. They need to review her history and record her symptoms, hypothesize potential factors that could have contributed to the onset of the meat allergy, record future tests ordered, and results as they are available.
4. (10 minutes) Allow the students to work in small groups (2-4) to complete as much of the chart as they can.
5. Circulate to check for understanding and remind them they have access to textbook glossaries should they need to clarify relevant vocabulary.
6. (5 minutes) When all student pairs have finished, go through the epidemiological report together, calling on student pairs to give answers.

*Teaching tip: The students do not have all of the information in this initial report. The purpose of this activity is to generate curiosity rather than identify a specific answer so it is ok if students get frustrated if you withhold information. The location for their hypotheses should be free from judgement and used to help drive student to student conversation, however, efforts to draw students back to evidence presented in the case study should be made by the teacher in order to reduce wild guesses that may lead students outside of the scope of the overall curriculum. Part 2 of this lesson is set up to be a review of macromolecule structure and function and biotechnology, since this lesson is designed to be taught as part of a unit on the immune system, that portion of the jigsaw represents the bulk of the new content.*

#### **ASSESSMENT SUGGESTIONS:**

- Participation grade may be given.
- Jigsaw note taking handouts can be collected at the end of the activity and graded for completion or accuracy.
- Exit Ticket:Formative Assessment=Exit ticket-Describe the relationship between the different classes of macromolecules and immune responses associated with the alpha gal meat allergy.

#### **RESOURCES/REFERENCES:**

There are a variety of scholarly journals and external resources (see background relevant literature) describing the ongoing research associated with this allergy. Additional articles providing an overview have been published by news organizations like [NPR:Red Meat Allergies Caused by Tick Bites On The Rise](#) or the final step in this curriculum requires students to read the scholarly journal article The alpha-gal story: Lessons learned from connecting the dots published in the Journal of Allergy and Clinical Immunology in March of 2015.

This case report is inspired by a true story heard through the Radiolab Podcast broadcast October 27th 2016 which can be accessed here: [Alpha Gal-10/27/16](#)

### **Lesson 1 Part 1: Meat No More– Narrative and Case Report (Student Worksheet)**

In this activity, you will take on the role of an epidemiologist. Review the narrative and patient's history and record her symptoms, tests ordered, and results as they are known. You will continue to fill in the epidemiological report as you move through the unit.

On **June 28th** the Williams family (Mom-Julie age 45, Dad-Greg-age 47, Son-William-age 13, Daughter-Claudia-age 16 and their trusted Basset Hound Molly) worked to pack up the campsite and load it into what was becoming an increasingly overcrowded vehicle. They had spent the last week hiking the trails, kayaking the rivers and exploring the many state parks of the central panhandle of the state of Florida. While Greg setup the GPS for the long trip back to Fort Myers, Florida, Julie negotiated Molly who insisted on travelling at her feet on car rides, after which her focus shifted to their annual 4th of July BBQ the following week.

**July 4th** started much as any typical day in South Florida, hot and humid. The plan for the family BBQ was to eat early to avoid the late afternoon thunderstorms that can be counted on to spoil any outdoor event during Florida summers. Greg and Julie tended to the grill (burgers, ribs, chicken breasts), while the kids played with their cousins and neighbors in the pool. Friends and family had brought all manner of side dishes and both Greg and Julie were convinced that they would soon run out of room in their now overfilled fridge. As expected, the rains came, but only after everyone had eaten their fill. After helping to clean up most of the guests departed and the William's family went about their evening routine; back to work tomorrow for both Greg and Julie and Claudia and now that Claudia had a freshly minted drivers license she was determined to look for a part time job, William (the youngest) however, would be off to day camp for the rest of the week, so everyone was off to bed around 9 o'clock. Later that evening Julie awoke feeling "not quite right". She felt flushed and warm and felt some mild abdominal pain. A look in the mirror, produced mild alarm as Julie's face was puffy, and she could see what appeared to be hives on her back. Slightly alarmed she awoke Greg, who seeing her symptoms trudged off to the all night pharmacy to acquire some over the counter anti allergy medication Claritin. Julie, slightly concerned because she had never had any serious allergic reactions in the past, took the recommended dosage of Claritin and tried to get some sleep.

The following morning (**July 5th**) Julie awoke, and although there were still some hives present, her swelling and rash had subsided. Relieved, she assumed the reaction was the result of a combination of too much sun and maybe something "off" in one of the many side dishes brought by people at the BBQ. No one else in the family had a similar reaction, so she made a mental note to call around to others that attended the BBQ to see if anyone had a similar reaction. A few calls throughout the day to family and friends identified her as the only one to have such a reaction, since she felt fine, she quickly forgot about it and got on with the rest of the week.

A few days later (**July 9th**) after what had been a routine week, the family finished cleaning up from a dinner of ribeye steaks, baked potato and fresh corn and went about their respective evening routines. The "not quite right feeling" returned shortly after dinner, but the this time here symptoms were much more severe. The few hives she had during her previous episode now covered her entire upper torso, her hands had swollen so much her wedding rings cut into her fingers, her heart pounded in her chest and she felt extremely light headed, as she headed for the

medicine cabinet, Greg got one look at her; before he could reach for his phone to call 911, Julie hit the floor.

*At the emergency department, Julie's vitals were logged on her chart: temperature of 98.8°F (37.1°C), heart rate of 110 beats per minute, blood pressure of 88/57 mmHg, and respiratory rate of 28 breaths per minute. The attending physician ordered an adrenaline shot (epinephrine) which stabilized Julie's vitals and she was admitted for further testing. Suspecting a viral or bacterial infection the attending physician ordered a complete blood culture: A complete blood cell (CBC) count revealed a white blood cell count of 6,900/μL (normal: 4,500--10,500/μL), a red blood cell count of 5.5 million/μL (normal: 5-6 million/μL). Hemoglobin levels were measured to rule out anemia: the patient's levels 15.2 g/dL (normal: 13.5-17 g/dL). Her evaluation included an unremarkable computed tomography (CT) scan of the head and a lumbar puncture. The patient's light-headedness resolved, and she was discharged after a 7.5-hour stay in the emergency department.*

On **July 12th**, Julie entered what she had eaten into her food journal. Suspecting that her recent trip to the emergency room had been prompted by a reaction to something she had eaten she had been diligently recording her meals and snacks ever since:

Date	Breakfast	Lunch	Dinner	Snacks
7/10/12	Bagel-Cream Cheese Strawberry Yogurt Granola	Turkey Sandwich (mayo, lettuce, tomato) Sunchips-Regular Iced Tea	Chicken Parmesan (tomato sauce, pasta, breaded chicken, mozzarella cheese)	Apple Popcorn
7/11/12	Egg and cheese biscuit Coffee	Chicken Caesar Salad Iced Tea	Chicken Tortillas, lettuce, tomato, cheddar cheese, rice and black beans	Granola Bar Banana Snickers Bar
7/12/12	Cheerios and Milk Coffee Toast and Peanut Butter	Roast Beef Sandwich (mayo, lettuce, tomato) Chef Salad (tomato, ham, turkey, cheddar cheese, romaine lettuce, ranch dressing)		Blueberry Yogurt

As Julie was preparing to leave work to pick up her son William she felt the onset of the symptoms she experienced earlier that week, not one to put herself or others in danger, she immediately asked a co-worker to contact her husband, Greg, and take her to the same nearby emergency room that she was admitted to on **July 9th**.

*Luckily the same attending physician was on duty when she was admitted and remembered her from her earlier visit. The visual symptoms were similar (hives, rash, swelling, low blood pressure) and the blood work came back similar as before. The attending physician, determined to take a more detailed history, followed up with Greg in the waiting room to expand on the food journal that Julie had kept, he focused on meals eaten the day of and the day before Julie's symptoms appeared. What did each day have in common? A meal the consisted of multiple servings of red meat. A meat allergy was unheard of by the attending physician, he consulted the literature and found evidence of patients presenting with similar symptoms in the Southeastern United States. In all cases the patients had eaten red meat several hours before their visit to the emergency room and all presented with what appeared to be an immune system driven allergic reaction. The appropriate diagnostic tool was an*

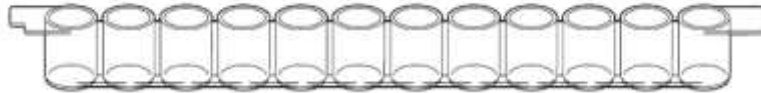
*ELISA assay of blood serum to look for antibodies specific to molecules unique to red meat. The physician drew samples of blood serum and sent them off to the lab for analysis.*

**EPIDEMIOLOGICAL REPORT**

Patient Case #: \_\_\_\_\_ Gender: Male  Female  Age: \_\_\_\_\_

Home address: \_\_\_\_\_ Recent travel: \_\_\_\_\_

Date	Symptoms	Test performed	Result	Diagnosis?
	<input type="checkbox"/> Chills or Fever <input type="checkbox"/> Hypertension (high BP) <input type="checkbox"/> Rash/Hives <input type="checkbox"/> Hypotension (low BP) <input type="checkbox"/> Headache <input type="checkbox"/> Elevated Heart rate <input type="checkbox"/> Joint/muscle pain <input type="checkbox"/> Low Heart rate <input type="checkbox"/> Light-headed          Other: _____			
	<input type="checkbox"/> Chills or Fever <input type="checkbox"/> Hypertension (high BP) <input type="checkbox"/> Rash/Hives <input type="checkbox"/> Hypotension (low BP) <input type="checkbox"/> Headache <input type="checkbox"/> Elevated Heart rate <input type="checkbox"/> Joint/muscle pain <input type="checkbox"/> Low Heart rate <input type="checkbox"/> Light-headed          Other: _____			
Provide additional relevant patient history here:				
Provided hypothesized factors contributing to condition here:				



**Illustrate ELISA Results Here**



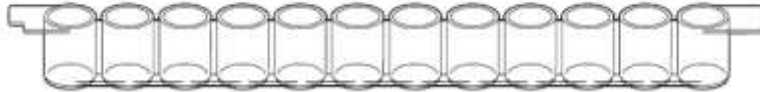
**Illustrate Results of Gel Electrophoresis Here**

**EPIDEMIOLOGICAL REPORT: Answer Key**

Patient Case #: 1 Gender: Male **X** Female Age: 45

Home address: Fort Myers, Florida Recent travel: Camping Trip in North Central Florida

Date	Symptoms	Test performed	Result	Diagnosis?
	<input type="checkbox"/> Chills or Fever <input type="checkbox"/> Rash/Hives <input type="checkbox"/> Headache <input type="checkbox"/> Joint/muscle pain <input type="checkbox"/> Light-headed and face <input type="checkbox"/> Hypertension (high BP) <input type="checkbox"/> Hypotension (low BP) <input type="checkbox"/> Elevated Heart rate <input type="checkbox"/> Low Heart rate Other: <u>Swelling in hands</u>	Blood Tests Hemoglobin CT Scan	Normal Normal Normal	Potential temporary allergic reaction of unknown cause) Patient recommended to keep food journal.
	<input type="checkbox"/> Chills or Fever <input type="checkbox"/> Rash/Hives <input type="checkbox"/> Headache <input type="checkbox"/> Joint/muscle pain <input type="checkbox"/> Light-headed <input type="checkbox"/> Hypertension (high BP) <input type="checkbox"/> Hypotension (low BP) <input type="checkbox"/> Elevated Heart rate <input type="checkbox"/> Low Heart rate Other: _____	ELISA	?	Possible meat allergy?
Provide additional relevant patient history here:				
Provided hypothesized factors contributing to condition here:				



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**Illustrate Results of Gel Electrophoresis Here**

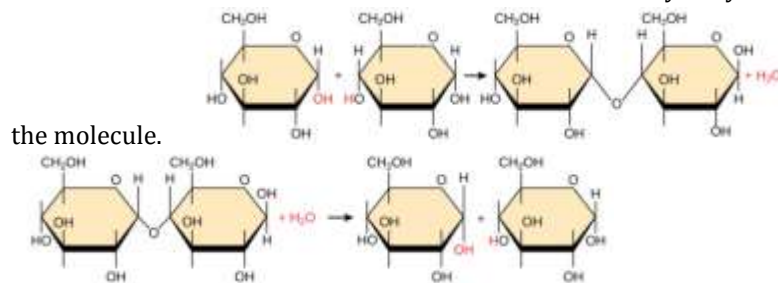
## Lesson 1 Part 2: Jigsaw

In this activity, you will work collaboratively with a team to build your background knowledge of biological concepts important to helping you with the epidemiological report you have completed as well as all future diagnostic techniques required to understand your eventual diagnosis. Work with your expert group to correctly organize your content cards to help you answer the guided questions on the student handout provided to you.

### Macromolecules

**A-Macromolecules** are large molecules composed of building blocks called monomers. Biologists typically identify 4 major categories of macromolecules: Carbohydrates, Lipids, Proteins, and Nucleic Acids.

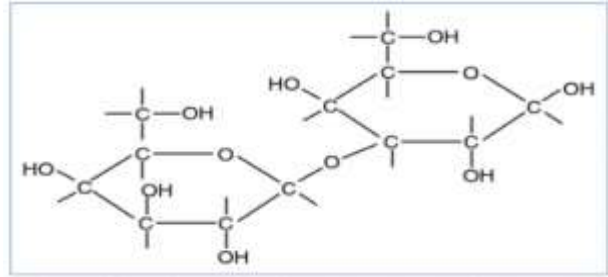
Macromolecules have several similarities: structurally they are all hydrocarbons (made up of carbon and hydrogen), and the metabolic reactions to construct the larger macromolecules from their smaller monomer subunits occur through dehydration reactions where a water molecule is removed, the breakdown of macromolecules into their smaller subunits occurs via a hydrolysis reaction, where water is added back into



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**B-Structural differences** in classes of macromolecules are often the result of the presence or absence of functional groups. Functional groups that play important roles in macromolecules include: Hydroxyl groups (**R-OH**) which because of their polar nature, can make molecules they are attached to more soluble in water, Carboxyl groups (**R-COOH**) which make their macromolecules behave as an acid in solution, Amino groups (**R-NH<sub>2</sub>**) which make their macromolecules behave as a base in solution, Phosphate groups (**R-PO<sub>4</sub>**) which also make their macromolecules behave as if they are acids, Sulfhydryl (**R-SH**), which are unique to certain proteins providing tertiary structural support, and methyl groups (**R-CH<sub>3</sub>**) which can play a role in DNA gene expression.  
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**C**-Carbohydrates are identifiable by the ratio of carbon:Oxygen:Hydrogen (1:2:1) and the suffix **-ose** at the end of their name. Most often these molecules are associated with energy storage molecules of glucose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>) used in cellular respiration by most heterotrophic organisms and made by most autotrophic organisms through photosynthesis. Other functions of carbohydrates are associated with the attachment to other macromolecules such as a protein to form a glycoprotein or a lipid to form glycolipids. These molecules are often found attached to plasma membranes and serve as mechanisms for cell to cell communication and identification. A particular carbohydrate associated with emerging red meat allergies, is the disaccharide (2 sugar monomers) Galactose-1,3-Alpha-Galactose, also known as Alpha Gal. This carbohydrate is common on the tissue cells of non-primate animals that are considered Red Meat (Beef, Pork, Venison etc.).

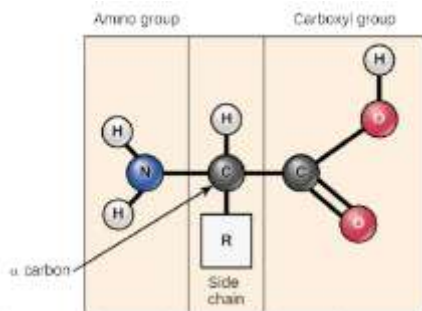


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**D**-Lipids, like carbohydrates are often comprised of carbon, hydrogen and oxygen atoms, but can be distinguished from them because they lack the 1:2:1 ratio typical of a carbohydrate. Lipids are typically non-polar and are less likely to dissolve in polar solvents such as water. Certain classes of lipids like steroids are important cell signalling molecules associated with the endocrine system that can elicit a variety of cellular responses throughout the body. Other lipids like phospholipids and their polar phosphate functional groups help to create the selectivity that the plasma membrane is responsible for.

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**E**-Proteins are identifiable because they are made up of the repeating amino acid monomers that contain the amino (NH<sub>2</sub>) functional group and the carboxyl functional group (COOH). There are 20 amino acids that can combine to make up a nearly endless combination when joined together via a dehydration reaction to form



proteins. Each of the 20 amino acids has a unique R group that influences the characteristics of the amino acid (polar, non-polar, electrically charged etc). Which will ultimately influence the final three dimensional structure of the protein. Proteins are the “workhorses” of living things, acting as enzymes, structural proteins, transport proteins, ligands, receptor proteins etc. Proteins are constructed based on the genetic sequence of specific genes in an organism's DNA. The enzyme that helps to construct the Alpha Gal carbohydrate that is associated with the meat allergy is Beta-galactosyl alpha 1,3 galactosyl transferase. The gene to create this enzyme is present in all mammals but is inactive in humans and other

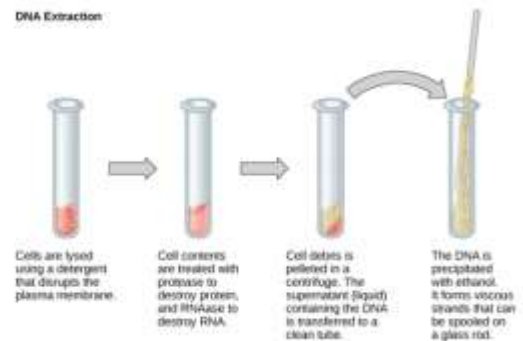
primates.

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**(F)**-Nucleic acids consist of DNA and RNA. DNA is located in the nucleus of eukaryotic organisms and contains the genetic instructions for the production of proteins. The production of proteins is dependent on the genetic sequence of DNA being converted into mRNA and then used at the ribosomes (which are constructed of rRNA), where tRNA molecules transport the appropriate amino acids for assembly into protein. Genes are sequences of the base pairs adenine, cytosine, thymine and guanine (a, c, t, and g), many genes are conserved across different groups of living things and can be used to identify evolutionary relationships by looking for similarities between gene sequences of different organisms.

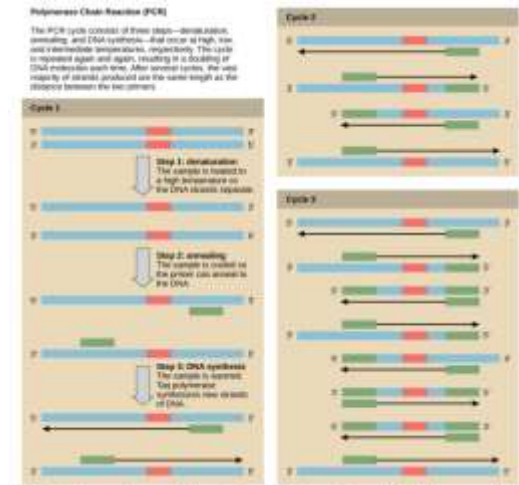
**Biotechnology**

**(A)**-DNA extractions are often the first step in an attempt to use DNA for diagnostic purposes. In eukaryotic organisms the DNA is confined to the nucleus of all of the organisms cells. Often times enzymes are used to digest tissues and cellular structures in order to suspend the DNA into a solution, several centrifuge steps allow for the separation of heavier cellular debris, like organelles, which will sink to the bottom to be removed while preserving the lighter molecular components like DNA and proteins. A series of washes are done with buffers to further purify the DNA and remove non DNA molecules from the sample.



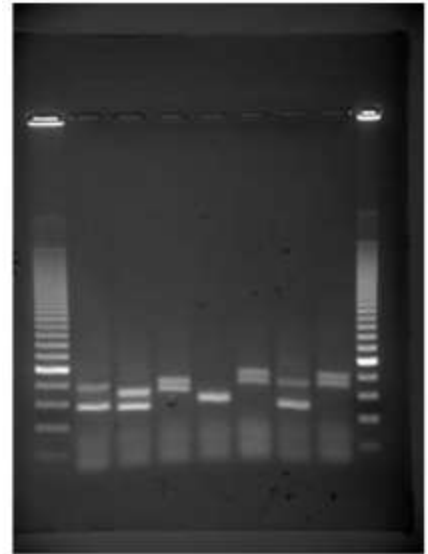
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**(B)**-After extracting the DNA it is important to amplify the amount of DNA as much as possible. The amount of DNA that was extracted is a finite amount, and if multiple diagnostic tests need to be run on an organism's extracted DNA, the extraction is followed up by a Polymerase Chain Reaction or (PCR) PCR exploits the structural components of the DNA double helix. The two halves of the DNA double helix are held together by weak hydrogen bonds. These bonds can be broken and reformed through a specific temperature cycle. Because DNA has a complimentary bonding pattern (A-T, C-G) DNA nucleotides, polymerase enzymes and DNA primers allows a single DNA molecule to be copied and amplified into tens of thousands in a matter of hours.



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**(C)**-In order to identify the size of DNA fragments contained within your DNA sample or to compare your DNA to that of another organism Gel Electrophoresis can be run. DNA samples are loaded into a collection of wells at one end of a gel. An electric current is passed through the gels (negative closest to the wells and positive furthest from the wells). Since DNA is a negatively charged molecule, the electrons that make up the electrical current actively push the DNA fragments through the gel. Smaller fragments of DNA are able to move at a faster rate and will travel further distances in the gel, while larger fragments will travel slower and will stay closer to the wells. As the Gel is run the different fragments will separate out into a pattern. Reference wells can be loaded with either a DNA ladder that will produce gel lines at specific DNA base pair increments (50, 100, 150, 200...) to identify how big unknown samples fragments are, or a reference sample can be used as a base of comparison. If the unknown samples DNA bands line up in the sample place as the reference organisms DNA sample then it likely your unknown DNA comes from that organism or one closely related to it.



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**(D)**-While electrophoresis can tell you that you have similar DNA to other organisms or can indicate the size of your DNA fragments, they are unable to identify the specific sequence of A, C, T, and G base pairs that may make up those DNA fragments. DNA sequencing techniques like Sanger Sequencing, once again use DNA's structure to help identify the specific sequence of a DNA fragment. Normally a replicating DNA molecule will add nucleotides to five carbon sugar by using the 3'OH group. In Sanger sequencing the DNA nucleotides provided for replication are known as ddNTP's and lack that 3'OH functional group meaning that additional base pairs can not be added, as a result DNA replication terminates and the end of the DNA fragment can be identified as an A,C,T, or a G. Doing this over and over again and analyzing the results with computer software will produce an accurate complete gene sequence.

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**(E)**-Once a DNA fragment has been sequenced it can be compared to other known DNA sequences and the organisms that those segments came from. This is done using a Basic Local Sequence Alignment Test (BLAST) which will compare DNA sequences of two or more organisms and look for areas of similarity and differences. This technique can be used to identify unknown DNA samples to an organism or can be used to look for evolutionary relationships between organisms. The more similarities that exist between the two DNA samples the more closely related the organisms will be on an evolutionary diagram like a phylogenetic tree or cladogram. This technique is not limited to DNA, a similar technique can also be applied to amino acid sequences.

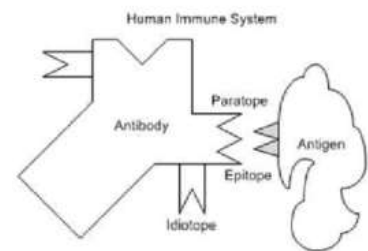
## Immune Response

**(A)**-The immune response is a system that is common to all animals. Although the complexities of that immune system vary widely between animals, sponges, the simplest animals have a few cell types designated to fight off foreign invaders, while complex mammals like humans have hundreds of different defenses against foreign invaders. However, the purpose of the immune system is the same, to maintain homeostasis by protecting the organism from foreign invaders. In humans the immune system is made up of two distinct stages, the innate immune system which is always present and defends against all pathogens, and the adaptive immune system which stimulates a specific response and also stores “information” about previous infections and illicit specific immune responses to future infections of the same pathogen.

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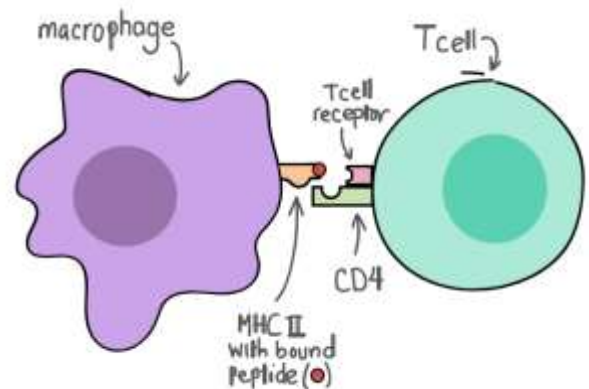
**(B)**-Extracellular “invaders” are called antigens, which often come in the form of a bacterial cell or virus. In some instances antigens may be associated with other parts of the environment like pet dander, pollen, even random chemicals that can activate the immune response as an allergic reaction. Specific parts of the invaders antigen called epitopes will interact with an immune molecule much like a lock and key which will start the immune systems defenses.

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**(C)**-The immune response is made up of a number of different cells completing a series of steps that ends with the response targeting the identified pathogen. Antigen presenting cells will “capture” and display the invading antigen on their cell surfaces via membrane receptors known as major histocompatibility complexes (MHC's) waiting for the next line of cells T-cells to arrive and initiate the next step of the immune response.

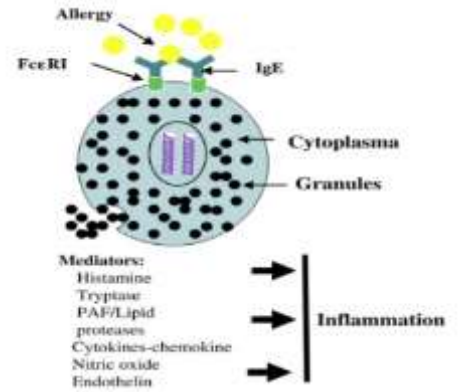
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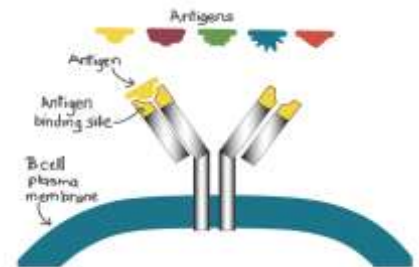
**(D)**-T-Helper cells are one of the most important cells in the immune systems arsenal. In the Alpha Gal/Red Meat allergy T-Helper cells will activate B-Cells, but in other immune responses they will also activate cytotoxic T Cells which will kill cells that have been compromised by the pathogen. T-helper cells will also create the memory component of the immune system which will dramatically increase the rate in which a future immune response can be mounted. T-helper cells are one of the cells that are compromised by viruses like HIV which make mounting an immune response against normally harmless pathogens difficult.

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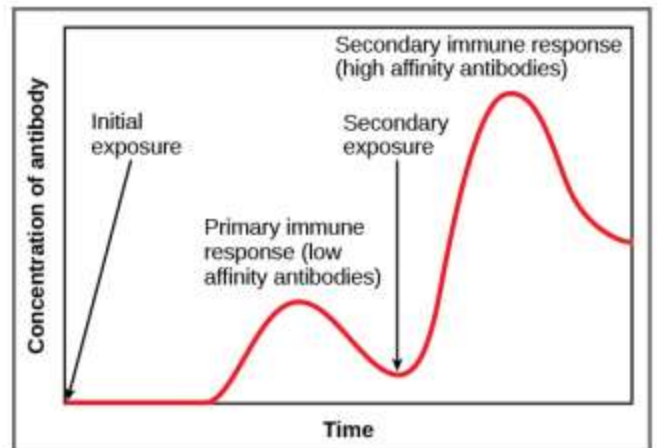
**(E)**-B-cells can be activated by T helper cells. One of the major roles of B-cells are to produce antibodies which increase the immune systems awareness of the pathogen. An antibody is essentially a receptor protein that will match an epitope region of the invading pathogen. In the case of the Alpha-Gal/red meat allergy, the antibody produced is known as immunoglobulin E (IgE). High concentrations of this specific antibody in a patient's blood serum can be used to diagnose red meat allergy.



**(F)**-In the case of an allergic reaction, an increase in antibodies like IgE can stimulate mast cells to secrete chemical messengers like histamine. The release of histamine by mast cells in turn will increase blood flow to the infected area and attract other specialized immune system cells which can lead to swelling, mucus production, rash, hives etc.



**(G)**-Finally the innate immune system has the ability to remember previous pathogens. By doing so specific steps in the immune system can be bypassed increasing the rate of the immune response and decreasing the time of illness. This memorized immune response is featured in the Alpha-Gal meat allergy. A pharmaceutical company running a clinical trial on a drug to treat cancer (Centuximab), notices unexpected allergic reactions of patients in the Southeastern United States. When they tested their blood serum they identified high volumes of the IgE antibody. Because the drug's molecular structure was very similar to the Alpha-Gal Epitope the immune system remembered the "pathogen" and initiated an immune response to the drug.



## Jigsaw Guided Questions-Student Handout

### Macromolecules

1. Identify the the 4 major categories of macromolecules and briefly describe how the process of dehydration synthesis and hydrolysis are related.
2. List the functional groups and identify which ones are relevant in the different classes of macromolecules.
3. How do you distinguish between carbohydrates and lipids? Provide a specific example of each class of macromolecule and list its function.
4. What is the role of proteins in most living things? How are the roles of nucleic acids and proteins interconnected?
5. The Alpha-Gal meat allergy is associated with carbohydrates, proteins and DNA. Describe the connection between these three classes of molecules and the Alpha-Gal allergy.

## Jigsaw Guided Questions-Student Handout

### Biotechnology

1. What is the purpose of using a centrifuge in the DNA extraction process. Where would you expect to find the DNA in relationship to the other parts of the cell? Why?
2. What is the purpose of the PCR step in a biotechnology application? How does the structure of DNA make this process possible?
3. What characteristic of the DNA molecule makes the process of Gel Electrophoresis possible. Briefly sketch out a sample Gel and hypothesize the location of a large, medium and small DNA fragments location in relationship to the starting well.
4. What is the purpose of DNA sequencing techniques and how do they relate to the BLAST process? What is the benefit of knowing a DNA sequence and being able to perform a BLAST?



## Jigsaw Guided Questions-Student Handout

### Immune System

1. What is the purpose of the immune system? How does the innate immune system compare to the acquired immune system?
2. What are antigens? Provide some examples and discuss how they are related to the term epitope.
3. Briefly discuss the role of the antigen, T-helper cells, B-cells, antibodies and Mast cells in generating an allergic reaction when the immune system is exposed to an epitope region of an antigen.
4. How is the immune system capable of decreasing its response time? How does this process relate to the drug Centuximab and the Alpha Gal/Red Meat allergy.

## Jigsaw Guided Questions-ANSWER KEY

### Macromolecules

1. Identify the the 4 major categories of macromolecules and briefly describe how the process of dehydration synthesis and hydrolysis are related.
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## Lesson 1 Optional: [Science Take Out-Pathogens, Antibodies and Vaccines](#) -Part A

Depending on your students needs and learning style preference, this science take out kit can be substituted for the immune system portion of the jigsaw. Provided below are the downloadable preview pages of part A of the kit. Each delivered kit comes with these handouts and a teacher answer key.

STO-138

### Pathogens, Antibodies, and Vaccines Teacher Information



#### Summary

How does the immune response protect against diseases caused by bacteria? Students model antigen-antibody interactions. They measure antibody levels to compare antibody levels following exposure to pathogens in unvaccinated and vaccinated children.

#### Core Concepts

- The immune system is responsible both for recovering from and preventing disease.
- Antigens are foreign proteins on the surfaces of pathogens.
- White blood cells produce antibodies and form memory cells.
- Antibodies attach to and destroy cells bearing antigens.
- The interaction between antigens and antibodies is specific.
- With first exposure to an antigen, the immune response is usually too slow to keep people from getting sick.
- With second exposure to an antigen, the immune response is rapid enough to prevent the disease.
- Vaccinations are injections of antigens that trigger the production of memory cells and antibodies.

#### Time Required

Three 40-minute class periods

#### Kit contains

- 3 foam balls to model bacteria
- Jewels (3 different shapes) to model antigens
- Glue dots
- Straws, clay, and rubber bands to model antibodies
- **Immunizations and the DTP Vaccine** brochure
- **Pertussis Antibody Test Instructions**
- 4 Pertussis Antibody Test Strips
- 4 tubes of simulated plasma samples

#### Teacher Provides

- Safety goggles
- Paper towels for clean up
- Colored pens/pencils (3 different colors)

#### Warning: Choking Hazard

This Science Take-Out kit contains small parts. Do not allow children under the age of seven to have access to any kit components.

# Kit Contents Quick Guide

**Immunizations and the DTP Vaccine**

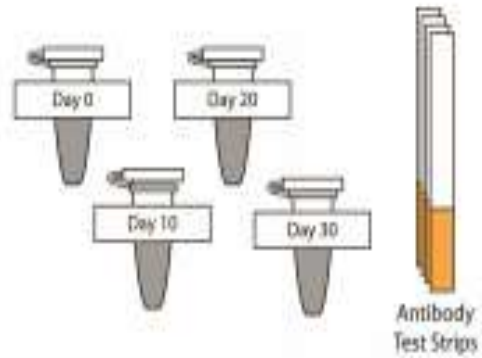


**IMMUNITY and IMMUNITY**

When you get vaccinated or exposed, your body produces disease-fighting substances called antibodies. These antibodies, or the white blood cells that carry them, usually stay in your body to prevent future infections, giving you some immunity.

**IMMUNIZATION and IMMUNITY**

Immunizations are also called vaccinations, shots, or needles. They contain dead or weakened pathogens, or parts of pathogens that trigger your body's immune system to produce antibodies that fight pathogens. Most vaccines need to be given more than once to work correctly.

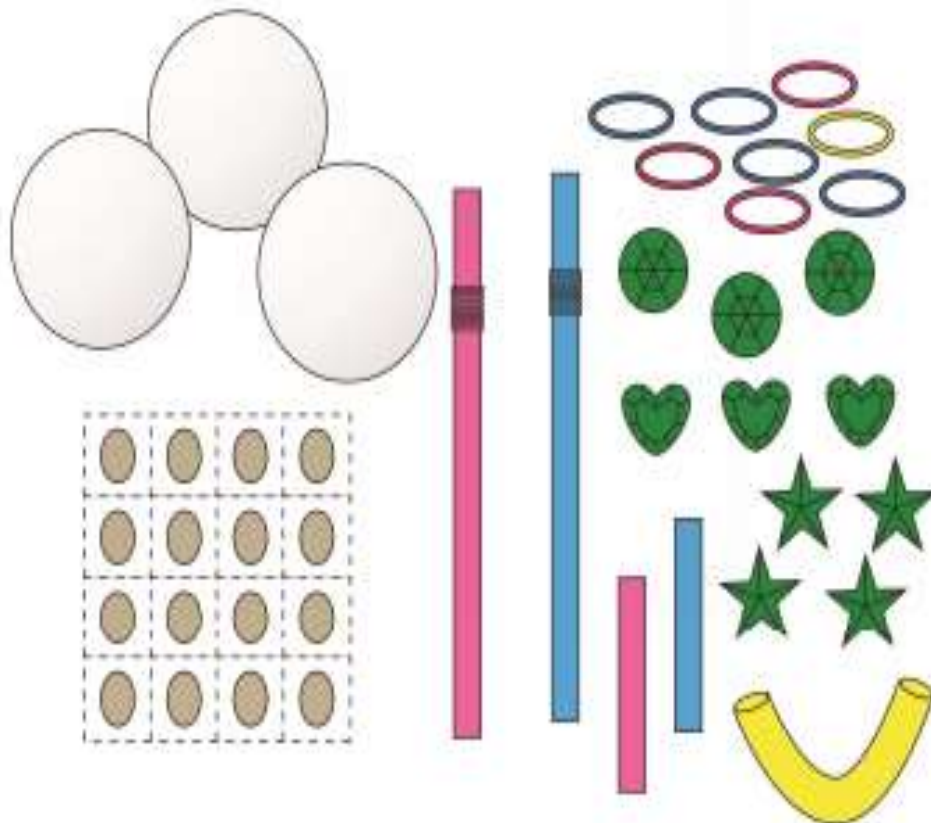


**Perforin Antibody Test Instructions**

Use the orange cap of an antibody test strip to draw blood. Allow to stand for about 10 minutes. Store eggs. Antibody Test Strips for next sample test period.

Use the color chart below to determine the relative concentration of perforin antibodies in the sample.

Relative Concentration of Perforin Antibodies						
0	1	25	50	100	200	50,000



# Pathogens, Antibodies, and Vaccines - Teacher Answer Key

## Part I: Modeling Pathogens and Antibodies

Three dangerous diseases:

- **Pertussis** (whooping cough) is caused by *Bordetella pertussis* bacteria
- **Diphtheria** is caused by *Corynebacterium diphtheria* bacteria
- **Tetanus** (lockjaw) is caused by *Clostridium tetani* bacteria

What happens when the body is invaded by the pathogens that cause these dangerous diseases?

**Pathogens** are disease causing microorganisms, such as bacteria, viruses, and fungi.

1. Use the information in the **"Immunizations and the DTP Vaccine"** brochure in your kit to complete the following chart.

Disease	Pathogen (bacteria, viruses, or fungi)	Contagious (yes or no)	3 Symptoms of disease	2 Body systems affected	Probability of death if infected
Diphtheria	<i>Bacteria</i>	Yes			___ in 10
Tetanus lockjaw	<i>Bacteria</i>	No			___ in 10
Pertussis whooping cough	<i>Bacteria</i>	Yes			___ in 10

## Bacteria cause pertussis, tetanus, and diphtheria

Pertussis, tetanus, and diphtheria are caused by pathogenic bacteria. You will use the materials in your kit to make models of these three bacteria.

- The three foam balls represent **bacteria**. Use a pen or marker to label the balls—pertussis, tetanus, or diphtheria.



### Three Types of Bacteria

- The three types of bacteria have different **proteins** on their surfaces. The adhesive jewels represent surface proteins on the bacteria. Use the glue dots to firmly attach three of the same type of jewels to the surface of each of the bacteria. *Note: Save the extra star jewel (pertussis) for use later.*



### Three Types of Surface Proteins on Bacteria

- How are the bacteria that cause pertussis, tetanus, and diphtheria different?



## Antigens

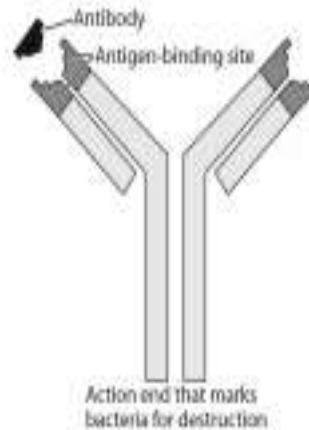
Humans do not make the proteins that are found on the surfaces of the bacteria that cause pertussis, tetanus, and diphtheria. So if these bacteria enter the human body, the bacterial proteins would be recognized as foreign proteins. **Antigens** are foreign proteins that your body does not normally contain.

When your body recognizes an antigen, it triggers an immune response that will destroy the bacteria that have this antigen. During the **immune response**, white blood cells of your immune system produce **antibodies**. Antibodies are proteins made by your body that bind (attach) to and destroy bacteria.

5. What is an antigen?
6. Which parts of your models represent antigens?
7. What type of cells makes the immune response?
8. What molecules are produced during an immune response to destroy bacteria?
9. Your body cells have surface proteins. Why don't you make antibodies against these surface proteins?

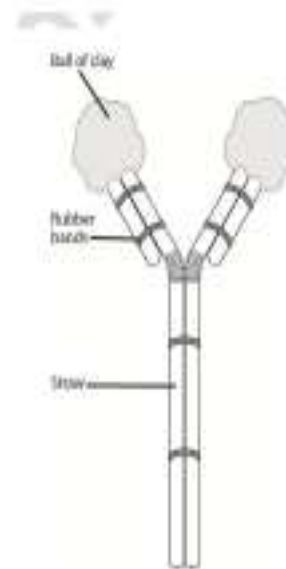
## Antibodies

During an immune response, white blood cells produce and release defensive proteins called **antibodies**. Each antibody molecule is a Y-shaped protein with two antigen binding sites on the ends. The antigen binding sites will bind to antigens on the surface of bacteria. The other end of the antibody is a “flag” that marks bacteria for destruction.



10. Use the four pieces of straw and small rubber bands to create a Y-shaped antibody molecule. Your model should look something like the diagram on the right.

11. Each antibody has two **antigen binding sites**. These specific antigen binding sites are **specific**. Specific means they have just the right shape to fit with one kind of antigen. You will make two antigen binding sites that can bind to the surface proteins (star jewels) on the bacteria that cause pertussis.



- Divide the strip of clay in half. Shape each half of clay into a ball.
- Make two pertussis antigen binding sites by pressing the star jewel (pertussis antigen) into the clay balls to make a pocket in the clay that will fit the pertussis antigen. Remove the star jewel to leave a pocket in the clay.
- Then, attach each of the balls of clay to the end of the straw. The pertussis antigen binding sites are on the ends of the Y shaped antibody, as shown in the drawing above. Make sure the star shaped pocket is facing out on the end of the antibody.
- Now you have a specific antibody that can bind to and destroy bacteria that cause pertussis.

12. Attach your model of an antibody to one of the antigens on the surface of the bacteria cell that causes pertussis.

13. How can you tell that the antibody you made is **specific** for the bacteria that cause pertussis?

14. How would an antibody for diphtheria be different from an antibody for pertussis?

15. How would an antibody for pertussis be similar to an antibody for diphtheria?

16. What happens to bacteria cells when antibodies attach to antigens on the surface of the bacteria?

17. Will the antibody model that you made be able to protect against the Bacteria X shown on the right? Explain why or why not.



18. Words that start with the same letters are easy to confuse. Use your creativity to develop a way to help other students remember the difference between an **antigen** and an **antibody**.

## Lesson 2

### LESSON 2 Part 1: Steps of an ELISA

**KEY QUESTION(S):** What is an ELISA? How is it used as a diagnostic test?

**OVERALL TIME ESTIMATE:** 15 – 40 minutes depending on prior knowledge

**LEARNING STYLES:** Visual, kinesthetic, and auditory

#### VOCABULARY:

Antibody

Antigen

ELISA

Primary antibody

Secondary antibody

**LESSON SUMMARY:** Student match diagrams with text descriptions to understand the steps of an ELISA. A common test used to detect if a patient has been exposed to a particular pathogen is called an ELISA (enzyme linked immunosorbant assay). This test takes advantage of the interactions between antigens and antibodies. Often compared to a lock and key, an antigen/antibody interaction is very specific. As you may recall from the Lesson 1, the antibody that is found in the blood serum in higher than normal concentrations is IgE.

#### STUDENT LEARNING OBJECTIVES:

The student will be able to...

- Sequence steps of an ELISA test
- Define ELISA
- Explain the use of an ELISA to aid in disease diagnosis
- Describe antigen/antibody interaction
- Diagram antigen/antibody interaction

#### AP Biology Curriculum Standards:

**Essential knowledge 2.D.4: Plants and animals have a variety of chemical defenses against infections that affect dynamic homeostasis**

**(B)** Mammals use specific immune responses triggered by natural or artificial agents that disrupt dynamic homeostasis

#### Next Generation Sunshine State Science Standards

**SC.912.L.14.52** Explain the basic functions of the human immune system, including specific and nonspecific immune response, vaccines, and antibiotics.

**SC.912.L.16.10** Evaluate the impact of biotechnology on the individual, society, and the environment, including medical and ethical issues.

#### MATERIALS:

Steps of an ELISA cards, cut (laminated for repeated use)

Steps of an ELISA student worksheet, per student pair (laminated for repeated use)

### BACKGROUND INFORMATION:

The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality-control check in various industries. In simple terms, in ELISA, an unknown amount of antigen is affixed to a surface, and then a specific antibody is applied over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and, in the final step, a substance containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal, most commonly a color change in the substrate.

Performing an ELISA involves at least one antibody with specificity for a particular antigen. The sample with an unknown amount of antigen is immobilized on a solid support (usually a polystyrene microtiter plate) either non-specifically (via adsorption to the surface) or specifically (via capture by another antibody specific to the same antigen, in a "sandwich" ELISA). After the antigen is immobilized, the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme, or can itself be detected by a secondary antibody that is linked to an enzyme through bioconjugation. Between each step, the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step, the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample.

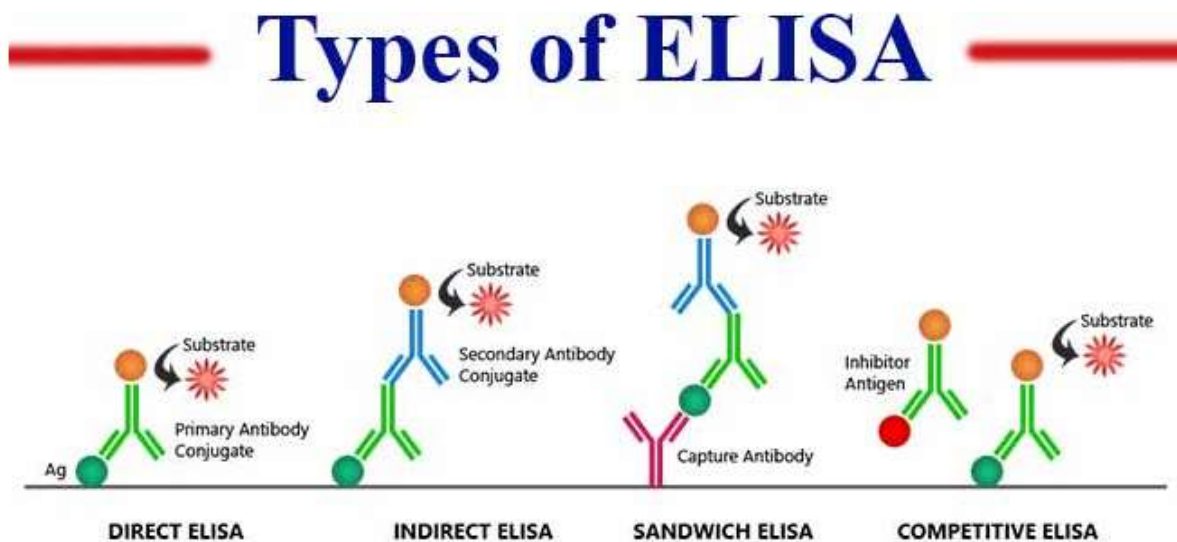


Image from Immunology Notes <https://immunologynotes.com/enzyme-linked-immunosorbent-assay-elisa/>

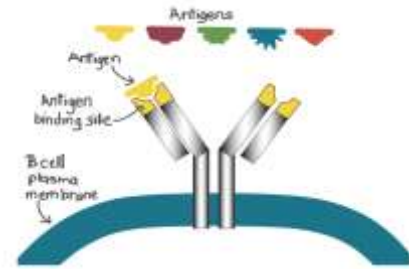
### ADVANCE PREPARATION:

Project the image of antigens and antibodies.

*Teaching tip: Make this into a file folder game. Affix the Steps of an ELISA student worksheet to the inside left and right sides of a file folder. Laminate for repeated use. Color copy the ELISA cards, laminate, and cut. Using small pieces of Velcro, place on side on the ELISA cards and the other in the center of each step on the worksheet.*

## PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. Review or introduce the following key points about antibodies and antigens:
  - Antigens are foreign molecules (carbohydrates/proteins/lipids etc.) which cause the immune system to generate antibodies.
  - Specific antibodies are produced for each antigen. They bind like a lock and key.
  - There are different antibodies produced by the human immune system (IgG, IgM, IgE, etc) but all have the same basic starting structure: a Y. At the top of the Y is the part that recognizes a specific antigen.
  - The part of the antigen that binds with the antibody is referred to as the epitope. In this picture, it is represented by the different shapes at the end of each different colored antigen.
  - Show the students that the tops of the antibody “Y” fits with certain epitopes on the antigens. Some antigens have multiple epitopes, so they are recognized by different antibodies (kind of like a back-up system). *The Science take out kit can also be supplemented here as a teacher demonstration (see optional activity in lesson 1)*
  - Tell students scientists have developed diagnostic assays that utilize the unique and specific binding properties of antibodies and antigens.
  - Introduce the idea that antibodies can serve as antigens as well and in diagnostic assays we create antibodies that recognize other antibodies as antigens or a protein which it is specific for.
  - Primary antibodies recognize the original antigen we are testing against. Secondary antibodies recognize the first (primary) antibody. Using both increases sensitivity.
2. Arrange students in pairs.
3. Distribute Steps of an ELISA cards and student worksheets to each pair.
4. Tell them to follow the directions on the worksheet.
5. (5-10 minutes) Allow student pairs to complete activity.
6. Review the steps together, clarifying as needed.
7. Show the video to reinforce how an ELISA is performed.



<http://www.youtube.com/watch?v=RRbuz3VQ100&feature=related>

### ASSESSMENT SUGGESTIONS:

Instructor can visually observe correct completion of the activity.

### RESOURCES/REFERENCES:

ELISA video: <http://www.youtube.com/watch?v=RRbuz3VQ100&feature=related>

## Lesson 2: Steps of an ELISA- Student Worksheet

A common test used to detect if a patient has been exposed to a virus such as HIV, Dengue, or West Nile is called an ELISA (**E**nzyme **L**inked **I**mmuno**S**orbant **A**ssay). This test takes advantage of the interactions between antigens and antibodies. Often compared to a lock and key, an antigen/antibody interaction is very specific. ELISA tests usually take place in plastic plates containing wells, or depressions.

Match the statements and images below to sequence the steps of an ELISA test.

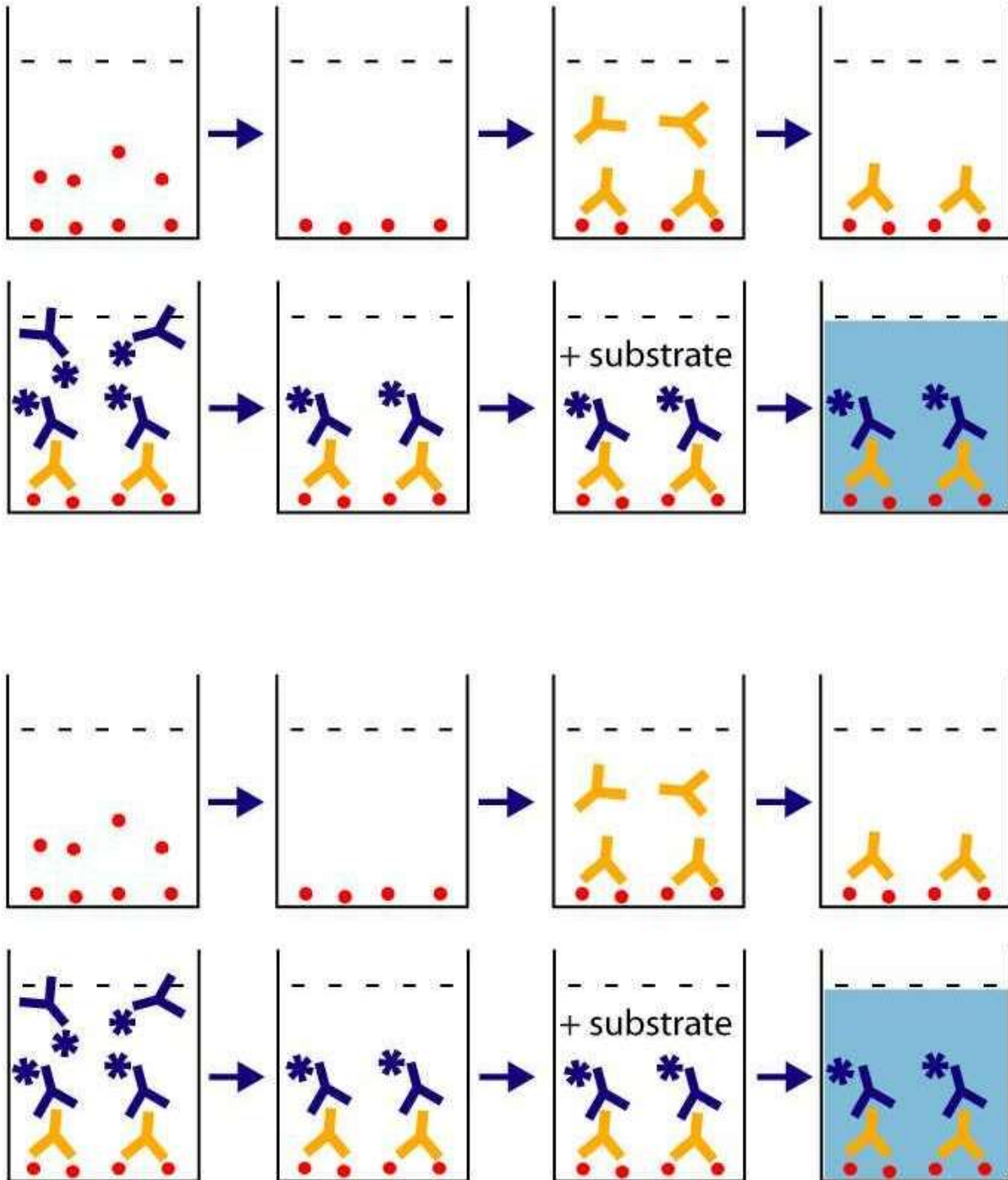
	Virus proteins (antigens) are added to wells of a 96-well plate.
	The antigens bind to the plastic, coating the bottom of the wells.
	The primary antibody is added to the well. In the case of the dengue ELISA, the primary antibodies (IgM) are from the patient's serum sample.
	Excess antibody is washed away, leaving only antibodies bound to the antigens behind. This wash removes excess antibodies that are unbound and prevents non-specific binding.

	<p>A secondary antibody is added to the wells. This antibody recognizes the patient IgM antibodies, bound to the antigens. The secondary antibody also has a colorimetric tag attached.</p>
	<p>Excess secondary antibody is washed away, leaving only secondary antibodies, bound to the patient IgM antibodies. This wash removes excess antibodies that are unbound.</p>
	<p>A substrate is added to the wells.</p>
	<p>Bound secondary antibody containing a colorimetric tag will cause a color change when exposed to the substrate. A color change indicates a positive reaction.</p>



## Lesson 2: Steps of an ELISA- cards

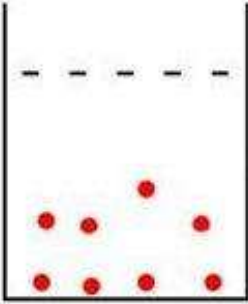
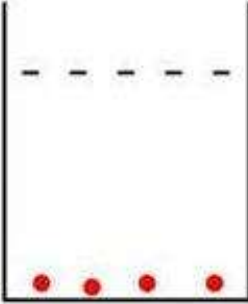
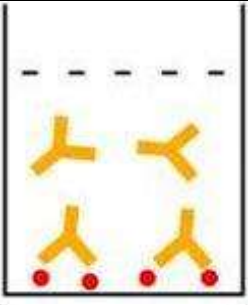
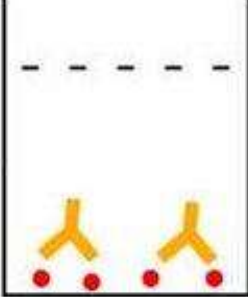
Make one set of eight cards for each group. Cut on both sides of arrows and between rows to separate into eight cards, each representing a step in the ELISA reaction.

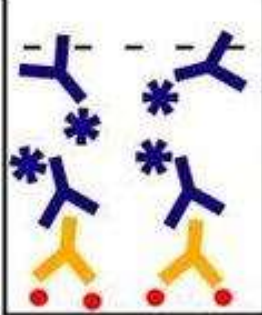
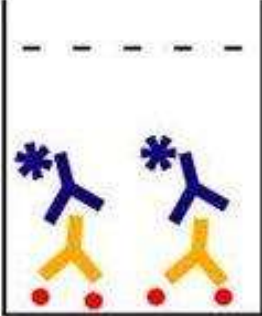
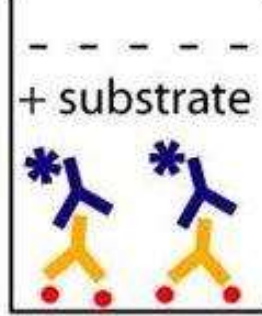



## Lesson 2: Steps of an ELISA- Teacher Answer Key

A common test used to detect if a patient has been exposed to dengue virus is called an ELISA (Enzyme Linked ImmunoSorbant Assay). This test takes advantage of the interactions between antigens and antibodies. Often compared to a lock and key, an antigen/antibody interaction is very specific. ELISA tests usually take place in plastic plates containing wells, or depressions.

Match the statements and images below to sequence the steps of an ELISA test.

	Virus proteins (antigens) are added to wells of a 96-well plate.
	The antigens bind to the plastic, coating the bottom of the wells.
	The primary antibody is added to the well. In the case of the dengue ELISA, the primary antibodies (IgM) are from the patient's serum sample.
	Excess antibody is washed away, leaving only antibodies bound to the antigens behind. This wash removes excess antibodies that are unbound and prevents non-specific binding.

	<p>A secondary antibody is added to the wells. This antibody recognizes the patient IgM antibodies, bound to the antigens. The secondary antibody also has a colorimetric tag attached.</p>
	<p>Excess secondary antibody is washed away, leaving only secondary antibodies, bound to the patient IgM antibodies. This wash removes excess antibodies that are unbound.</p>
	<p>A substrate is added to the wells.</p>
	<p>Bound secondary antibody containing a colorimetric tag will cause a color change. A color change indicates a positive reaction.</p>

## **LESSON 2-Part 2: Testing for Alpha Gal Antibodies**

**KEY QUESTION(S):** Does Julie, our patient, test positive for antibodies specific to the Alpha Gal carbohydrate?

**OVERALL TIME ESTIMATE:** 45 minutes

**LEARNING STYLES:** Visual and kinesthetic

### **VOCABULARY:**

Antibody

Antigen

ELISA

Primary antibody

Secondary antibody

**LESSON SUMMARY:** Using a commercial classroom-friendly ELISA kit, students will test the patient serum sample for the presence of dengue antibodies, and record their results on the epidemiological report. A simulated version is also presented.

### **STUDENT LEARNING OBJECTIVES:**

- Perform an ELISA test
- Explain the use of biotechnology to diagnose disease
- Recognize that an ELISA is an antibody-based test rather than nucleic acid
- Explain the steps of an ELISA
- Propose other uses of an ELISA

### **AP Biology Curriculum Standards:**

**Essential knowledge 2.D.4: Plants and animals have a variety of chemical defenses against infections that affect dynamic homeostasis**

**(B)** Mammals use specific immune responses triggered by natural or artificial agents that disrupt dynamic homeostasis.

**Science Practice 4:** The student can plan and implement data collection strategies appropriate to a particular scientific question

**Science Practice 5:** The student can perform data analysis and evaluation of evidence

### **Next Generation Sunshine State Science Standards**

**SC.912.L.16.10** Evaluate the impact of biotechnology on the individual, society, and the environment, including medical and ethical issues.

**SC.912.L.16.11** Discuss the technologies associated with forensic medicine and DNA identification, including restriction fragment length polymorphism (RFLP) analysis.

**SC.912.N.1.1** Define a problem based on a specific body of knowledge, for example: biology, chemistry, physics, and Earth/space science...

**MATERIALS:**

*If performing the authentic ELISA, this curriculum recommends BioRad. Other companies also have classroom-friendly ELISAs, but the instructions provided here are specific to BioRad.*

ELISA test (BioRad's Biotechnology Explorer ELISA Immunoexplorer Kit Catalog # 166-2400EDU Protocol III – Antibody test. All necessary consumables are included in the BioRad kit.)

**OR**

*If performing the simulated ELISA, you will need the materials listed below:*

Fluorescent ink pen

12-well microplate strips

Assorted 1.5 or 2.0ml microfuge tubes

Microfuge racks

Disposable transfer pipets

P200

Disposable tips, 20-200ul

Clear or white unscented soap

Cups or small beakers

UV lights

**BACKGROUND INFORMATION:**

General ELISA background information can be found in the preceding lesson, as well as in the BioRad Laboratory Manual to accompany this experiment.

There are several different types of ELISA. For our meat allergy example, we are indirectly measuring the presence of IgE antibodies specific to the carbohydrate Galactose, 1,3-alpha-Galactose (Alpha Gal) in the patient's serum by capturing antibodies. The steps of an "indirect" ELISA follow the mechanism below:

1. A buffered solution of the antigen to be tested for is added to each well of a microtiter plate, where it is given time to adhere to the plastic through charge interactions.
2. A solution of non-reacting protein, such as bovine serum albumin or casein (non-fat milk powder is sometimes used), is added to block any plastic surface in the well that remains uncoated by the antigen.
3. Next the primary antibody is added, which binds specifically to the test antigen that is coating the well. This primary antibody could also be in the serum of a donor to be tested for reactivity towards the antigen.
4. Afterwards, a secondary antibody is added, which will bind the primary antibody. This secondary antibody often has an enzyme attached to it, which has a negligible effect on the binding properties of the antibody.
5. A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme. The color change shows that secondary antibody has bound to primary antibody, which strongly implies that the donor has had an immune reaction to the test antigen. This can be helpful in a clinical setting, and in R&D.
6. The higher the concentration of the primary antibody that was present in the serum, the stronger the color change. Often a spectrometer is used to give quantitative values for color strength.

IgM antibody capture ELISA (MAC-ELISA) format is most commonly employed in diagnostic laboratories and commercially available diagnostic kits. The assay is based on capturing human IgM antibodies on a microtiter plate. Galactose-1,3-alpha-Galactose (Alpha Gal) is first coated on the plate, followed by the addition of the patient serum sample containing IgE antibodies against Alpha Gal (primary antibody). To detect the bound IgE antibodies, anti-human-IgE antibody (the secondary antibody) is added to the plate. The enzyme-linked anti-human antibody will bind to the patient IgE. Once substrate is added, the enzyme is released causing a color change.

**ADVANCE PREPARATION:**

Copy Student ELISA Procedure for each student or student pair.

All directions for performing the ELISA can be found in the instruction manual which accompanies the BioRad kit and are not duplicated here. If using the BioRad ELISA, please follow the preparation instructions included in the kit.

The simulation instructions presented here are modeled after the BioRad kit, Protocol III. Therefore, whether the students are performing the authentic BioRad ELISA or a simulation, they will follow the same steps.

**Modified ELISA: Simulation Advance Preparation**

1. Prepare the ELISA plates.  
If using 12-well microplate strips, use a Sharpie or other permanent marker to number the wells at the top 1-12. If using 96-well plates, they should come with columns and rows marked.
2. Using a fluorescent ink pen, “paint” the outside bottom of wells 1-3 (positive serum) and wells 7-9 (patient serum). Allow to dry prior to use.
3. Prepare student station reagents using the chart below. Note: This provides quantities for 8 student workstations, each with 2-4 students. To allow students to work in smaller groups, but without increasing prep time aliquoting reagents, two student groups (2 microstrip plates) can use 1 set of reagents.

<b>Tubes (number needed)</b>	<b>Description</b>	<b>Label</b>	<b>Contents (Each Tube)</b>
Violet tubes, 8	Positive controls	+	0.5ml water
Blue tubes, 8	Negative controls	-	0.5ml water
Green tubes, 8	Purified antigen	AG	1.5ml water
Orange tubes, 8	Secondary antibody	SA	1.5ml water
Brown tubes, 8	Enzyme substrate	SUB	1.5ml water
Yellow tubes, 8	Patient sample	PAT	0.25ml water

3. Prepare wash buffer  
Add 5ml clear or white unscented dish soap to 1000 ml water. Mix well.  
Aliquot 50ml wash buffer per student group (Beakers, conical tubes, or cups work well.)
4. Assemble student workstations, or have students collect the items below from a common station.

<b>Item (Label)</b>	<b>Contents</b>	<b>Number per station</b>	<input checked="" type="checkbox"/>
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Yellow tube (PAT)	Patient sample (0.25ml)	1	
Violet tube (+)	Positive control (0.5ml)	1	
Blue tube (-)	Negative control (0.5ml)	1	
Green tube (AG)	Purified antigen (1.5ml)	1	
Orange tube (SA)	Secondary antibody (1.5ml)	1	
Brown tube (SUB)	Enzyme substrate (1.5ml)	1	
Beaker of wash buffer		1	
12-well microplate strip		1	
Disposable transfer pipette		7 (only 1 needed for wash buffer if using P200)	
20-200ul micropipette		1 (if available)	
20-200ul tips		1 box (if available)	
Stack of paper towels		1	

#### Implementation tips:

- Use P200 if available to add samples to the wells.
- Use disposable pipettes to add the wash buffer.
- Ensure students know how to use both the adjustable volume pipette as well as the disposable pipettes. Bubbles are not friendly in this experiment, and improper use of the pipettors has led to many wells bubbling over.
- Use absorbent towels. The brown paper towel standard in many schools does not adequately absorb liquid, causing samples to splash back and contaminate adjacent wells. This isn't a problem with the simulation, but is with an actual ELISA.
- Avoid rehydrating the antibodies, particularly the secondary antibody, until just prior to use.
- If possible, when performing the actual ELISA, keep all solutions and reagents cold until use.

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

The procedure is well written in the BioRad manual.

Read or provide copies of the continuation of case report and remind students to record results in their epidemiological report. For convenience, the continuation of the case report is included at the top of the Student ELISA Procedure.

**Story cont.**

*During the patient's second visit to the emergency room the attending physician, took a more detailed history and reviewed the notes made in patient's food journal. Recognizing a pattern of allergic reactions on days in which the patient consumed meat, the physician consulted the relevant literature and learned of similar cases of meat allergy associated with a specific carbohydrate called Galactose-1,3-Alpha-Galactose in patients throughout the southeastern United States. The other cases presented with increased immunoglobulin E (an antibody associated with allergic reactions) in their blood serum. As a result the physician ordered an ELISA test which identifies the presence or absence of specific antibodies in the blood stream.*

**ASSESSMENT SUGGESTIONS:**

BioRad includes focus and review questions which can be collected for assessment.

Modifications:

- Could modify the "script" of the Science Take Out Kit (optional lesson 1) to support the ongoing narrative.
- For advanced classes, teachers may consider extending the lesson to include a quantitative analysis such as spectrometer results
- As this unit is written now, there is only one patient to test, and she is positive, other cases can be simulated by incorporating patient statistics from the other states where the Alpha Gal Meat Allergy is common.



## Student ELISA Procedure

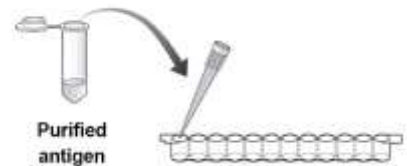
### *Our story continues.*

During the patient's second visit to the emergency room the attending physician, took a more detailed history and reviewed the notes made in patient's food journal. Recognizing a pattern of allergic reactions on days in which the patient consumed meat, the physician consulted the relevant literature and learned of similar cases of meat allergy associated with a specific carbohydrate called Galactose-1,3-Alpha-Galactose in patients throughout the southeastern United States. The other cases presented with increased immunoglobulin E (an antibody associated with allergic reactions) in their blood serum. As a result the physician ordered an ELISA test which identifies the present or absence of specific antibodies in the blood stream.

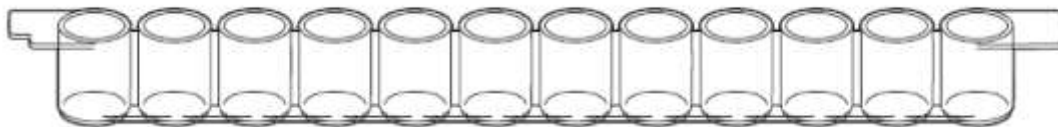
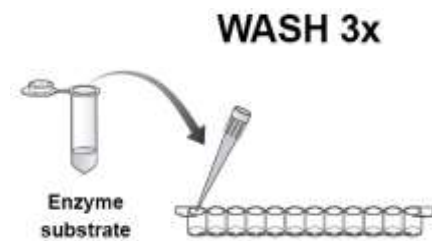
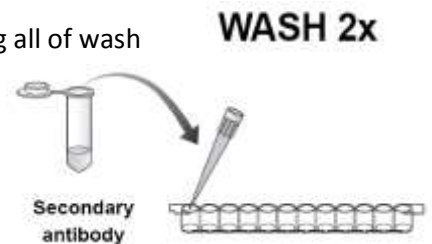
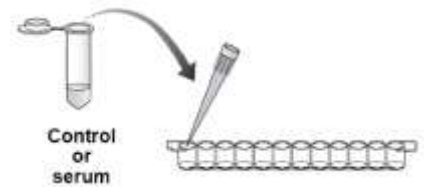
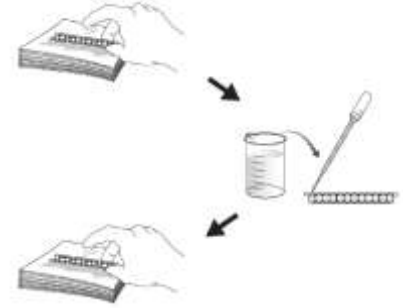
1. Review the student workstation checklist to ensure you have all needed reagents and supplies.

Item (Label)	Contents	Number per station	✓
Yellow tube (PAT)	Patient sample (0.25ml)	1	
Violet tube (+)	Positive control (0.5ml)	1	
Blue tube (-)	Negative control (0.5ml)	1	
Green tube (AG)	Purified antigen (1.5ml)	1	
Orange tube (SA)	Secondary antibody (1.5ml)	1	
Brown tube (SUB)	Enzyme substrate (1.5ml)	1	
Beaker of wash buffer		1	
12-well microplate strip		1	
Disposable transfer pipette		7 (only 1 needed for wash buffer if using P200)	
20-200ul micropipette		1 (if available)	
20-200ul tips		1 box (if available)	
Stack of paper towels		1	

2. Label wells 1-3 with a + (positive); label wells 4-6 with – (negative); label wells 7-9 with Pat (patient).
3. Use a fresh pipette tip to transfer 50ul of the purified antigen (AG) into wells 1-9 of the microplate strip.
4. Wait 5 minutes for the purified IgE Galactose-1,3-Alpha-Galactose antigen to bind to the plastic wells.
5. Wash:



- a. Tip the microplate strip upside down onto the paper towels, and tap the strip a few times upside down. Make sure to avoid splashing sample back into wells.
  - b. Discard the top paper towel.
  - c. Use your transfer pipette to fill each well (1-9) with wash buffer, taking care not to spill over into neighboring wells. Note: the same transfer pipette is used for all washing steps. Be sure to only draw up wash buffer, and not the contents of the wells.
  - d. Tip the microplate strip upside down onto the paper towels and tap.
  - e. Discard the top 2-3 paper towels.
6. Repeat wash step 5
  7. Use a fresh pipette tip to transfer 50ul of the positive control (+) into wells 1-3.
  8. Use a fresh pipette tip to transfer 50ul of the negative control (-) into wells 4-6.
  9. Use a fresh pipette tip to transfer 50ul of the patient serum (PAT) into wells 7-9.
  10. Leave wells 10-12 empty.
  11. Wait 5 minutes for the antibodies to bind to their targets.
  12. Wash the unbound primary antibody out of the wells by repeating all of wash step 5 two times. (Wash twice.)
  13. Use a fresh pipette tip to transfer 50ul of secondary antibody (SA) into wells 1-9 of the microplate strip.
  14. Wait 5 minutes for the antibodies to bind to their targets.
  15. Wash the unbound secondary antibody out of the wells by repeating wash step 5 three times. (Wash three times.)
  16. Use a fresh pipette tip to transfer 50ul of enzyme substrate (SUB) into wells 1-9 of the microplate strip.
  17. Wait 5 minutes. Observe and record the results on your epidemiological report.



### **LESSON 3: GIS Modelling-Making Connections**

**KEY QUESTION(S):** How were scientists able to connect the dots between the alpha gal red meat allergy and the Lone Star Tick as a potential “vector”?

**OVERALL TIME ESTIMATE:** 45 minutes

**LEARNING STYLES:** Visual, verbal

**VOCABULARY:**

**LESSON SUMMARY:** Using geographical incidences of alpha gal red meat allergy and occurrences of an allergic reaction the cancer drug Cetuximab (similar epitope region to Galactose-1,3-Alpha-Galactose) students will look for similarities of tick distribution in the United States in an effort to correctly identify the Lone Star tick (*Amblyomma americanum*) as the vector for the increased sensitivity to both the Alpha Gal carbohydrate and the Cetuximab cancer drug.

**STUDENT LEARNING OBJECTIVES:**

The student will be able to...

- Interpret data and incorporate it into a map of the United States
- Analyze the distribution of tick species and determine a relationship to a specific vector for the allergy
- Identify distinguishing characteristics of the different tick species endemic to the United States
- Identify pathogens (other than alpha gal) that are associated with the tick species of the United States.

**STANDARDS:**

#### **AP Biology Curriculum Standards**

**Science Practice 1:** The student can use representations and models to communicate scientific phenomena and solve scientific problems

**Science Practice 4:** The student can plan and implement data collection strategies appropriate to a particular scientific question

#### **Next Generation Sunshine State Standards**

**SC.912.L.14.6** Explain the significance of genetic factors, environmental factors, and pathogenic agents to health from the perspectives of both individual and public health

**SC.912.N.1.1** Define a problem based on a specific body of knowledge, for example: biology, chemistry, physics, and Earth/space science

**MATERIALS:**

Patient Data Sets (laminated for extended use)

Poster Size Maps of the United States (laminated for extended use)

Maps of Tick Distribution (laminated for extended use)

Dry Erase Markers or Sticky Note Flags

Optional printed background information on tick species from CDC/University of Florida Web Sources

## **Background Information:**

The identification of the Lone Star Tick as a potential vector for the alpha gal red meat allergy resulted from some sleuthing by researchers. Adverse allergic reactions to the a drug designed to treat cancer were popping up in a clinical trial. What was curious is that the majority of the cases were limited to the Southeastern United States. At the same time an increasing number of patients were presenting with a red meat of unknown cause. What tied the two observations together were their geographic location, again primarily in the Southeastern United States, as well as the lifestyle (active, outdoor recreation) of the patients presenting with these symptoms, active Recognizing a potential pattern between the distribution of these two reactions and the known distribution of tick species (specifically the Lone Star Tick) allowed scientists to connect the dots between all of the observation and begin to identify the molecular pathway associated with the immune response.

## **Advanced Preparation:**

Electronic copy of patient data sets for each student (technology version) or copies of patient data sets for pen/paper version (laminated for extended use)

Computer with access to internet and the google map program (technology version) or a single large copy of a map/group or smaller map for individual use (laminated for extended use)

Color maps of tick disease distribution (laminated for extended use)

Enough post it flags (two colors to distinguish between drug allergy and red meat allergy) or colored dry erase markers if doing the pen and paper version.

Computer access or hard copies of Tick background

To familiarize yourself and your students with how to plot points on a google map, please review this short tutorial.

<https://support.google.com/mymaps/answer/3024454?hl=en&co=GENIE.Platform%3DDesktop>

## **Procedure and Discussion with Time Estimates**

### **Part 1-45 minutes-Electronic Version**

1. (5 minutes) Tell the students that the case of Julie’s red meat allergy caught the attention of a medical geographer at the local university who began to look at other incidences of this strange allergy. Have the students read the background on what a medical geographer does and the data set they will be using during this part of the lesson. Review any questions the students may have regarding the background information.
2. (15 minutes) If working on the independent technology based version:
  - a. If working on the independent version of the assignment have students load the maps.google.com home page. (students need a google account to create personalized maps)
  - b. Students should click on “Your Places” and then “Maps” and finally “create map”.
  - c. Students can copy and paste the GPS coordinates provided on the electronic data table in order to drop appropriate pins to track the occurrences of the alpha gal meat allergy. Remind them that they should change the color (right click on the pin and fill it with a new color) to differentiate between the drug allergy and the red meat allergy.
3. (15 minutes) If working on the collaborative pen/paper based version:

- a. Arrange students in groups (2-4 students per group depending on availability of supplies)
- b. Tell the students to work as a team to identify the location of both alpha gal red meat allergy occurrences and the allergic reactions to the cancer drug. Students should distinguish allergic reactions (red meat vs. drug) with different colored sticky note flags or marks with their dry erase marker.
4. (10 minutes) Have the students read the second part of the medical geographers analysis that relates to the distribution of tick species in the United States. Have students review the maps of the tick related pathogen cases throughout the United States to identify the likely species responsible for both the drug allergy and the red meat allergy.
5. (5 minutes) After students have narrowed down or hypothesized the potential tick, have students read the final part of the medical geographers analysis and have students review the “picture” taken of the tick retrieved from the patient’s dog to confirm the tick that likely lead to the red meat allergy as the Lone Star Tick (*Amblyomma americanum*)
6. (10 minutes) Provide students with the background either by directing them to the either of the links below or by printing out the relevant information attached later in this lesson)
  - a. [http://entnemdept.ufl.edu/creatures/urban/medical/lone\\_star\\_tick.htm](http://entnemdept.ufl.edu/creatures/urban/medical/lone_star_tick.htm)
  - b. <https://www.cdc.gov/lyme/resources/TickborneDiseases.pdf>

**Teaching Tip:** Since GIS is a computer based field of research the activity has been optimized to work with the google map application. The GPS coordinates for the occurrences of the alpha gal allergy have been modified slightly from the source material to optimize the overlap with the Lone Star Tick associated pathogens. If you utilize the pen/paper version of the activity recognize that there may be some discrepancies in the distribution of the allergy as the students will interpret the geographic data differently (this can generate an opportunity for discussion about the role of technology and accuracy when forming conclusions). This can also be done as a whole class exercise if you have access to a smartboard style projection system. Students could each take responsibility for a particular patient’s location and plot it appropriately on a projected map for the entire class. It should also be noted that the “scenario” presented here greatly exaggerates the speed at which data collection, analysis and conclusions can be drawn. This point could become part of the post activity discussion as students realize that observations, analysis and conclusions take time and require a great deal of collaboration.

### Assessment Suggestions:

- Participation grade may be given
- Formative assessment in the form of discussion questions with each group

### Resources/References

The incidence data presented in the student data table was modified from:

Platts-Mills, T. A. E., & Commins, S. P. (2013). Emerging Antigens Involved in Allergic Responses. *Current Opinion in Immunology*, 25(6), 769–774. <http://doi.org/10.1016/j.coi.2013.09.002>

Chung, C.H., Mirakhur, B., Chan, E., & others (2008). Cetuximab-Induced Anaphylaxis and IgE Specific for Galactose-  $\alpha$ -1,3-Galactose. *New England Journal of Medicine*, 358(11), 1109-1117.. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2361129/pdf/nihms-45035.pdf>

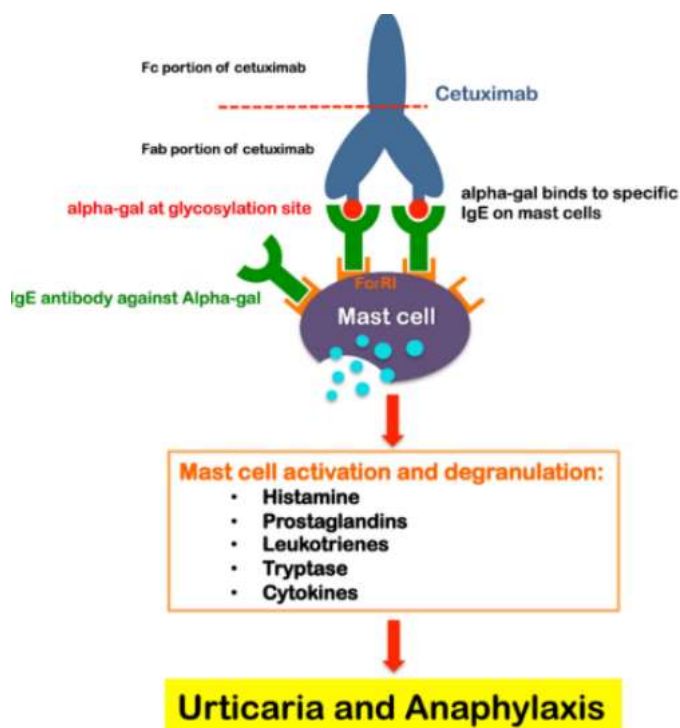
## Lesson 3

### Lesson 3: Part 1, Step 1: GIS Modelling-Making Connections

In this activity you will take on the role of a medical geographer. Review the short narrative discussing the field of medical geography, the tools that they use. Your responsibility will be to accurately map the cases of either the patients presenting with the Alpha Gal red meat allergy or the allergic reaction to the cancer treatment drug currently in clinical trials.

*Word had spread quickly through the hospital where our patient Julie was admitted. It is not often that hospital staff have the opportunity to investigate a new or emerging illness. A call was placed from a hospital staff member to the local university, where the hospital staff was quickly put in contact with the department of Geography. Thinking this was a mistake, the hospital staff member protested and asked to be connected to staff of the medical school. The professor on the other end politely explained that they were actually an interdisciplinary office and their area of expertise was medical geography. Their offices and labs used powerful computer models and simulations created using Geographic Information Systems that map occurrences and changes in many different environmental factors including emerging pathogens. The staff in the lab were going to access the medical literature in hopes of using their GIS systems to locate a potential pattern in the occurrences associated with this strange allergy.*

*A review of the literature by the staff in the medical geography department yielded two data sets. The first described the occurrences of what was being identified as the Red Meat Allergy tied to the Alpha Gal carbohydrate, the second was incidences of a drug reaction. The drug was designed to treat cancer. Medical researchers reviewed the immune response pathway and identified that the portion of the reaction that was generating the immune response was a region very similar in structure to the Alpha-Gal carbohydrate (see figure below):*



1. Use the patient information provided on the back of this page and the map you have been given to identify the locations where the drug allergy or the red meat allergy have occurred.

2. Use the different colored sticky note arrows or dry erase markers to differentiate between the two different allergic reactions.

3. When you have completed your map review it with your instructor where you will be provided with further instructions.

**Data Table for Independent Google Map Version**

	<b>Allergy</b>	<b>Lat N, Long W</b>		<b>Patient Diagnosis</b>	<b>Lat N, Long W</b>		<b>Patient Diagnosis</b>	<b>Lat N, Long W</b>
1	CDA	35.64123, -82.15513	30	RMA	35.49645, -82.24365	59	RMA	37.99616, -79.43115
2	CDA	35.37311, -78.81657	31	RMA	36.03133, -81.05712	60	RMA	37.89219, -78.44238
3	CDA	34.79823, -77.23422	32	RMA	35.5501, -80.41992	61	RMA	37.05517, -80.63964
4	CDA	35.7141, -78.90342	33	RMA	36.01356, -81.27685	62	RMA	36.93233, -80.92529
5	CDA	36.20946, -81.73832	34	RMA	35.26356, -83.71582	63	RMA	37.00255, -81.60644
6	CDA	36.05348, -80.81419	35	RMA	36.4743, -76.42089	64	RMA	36.84446, -81.23291
7	CDA	34.37971, -78.2666	36	RMA	36.45663, -76.90429	65	RMA	26.43122, -81.91406
8	CDA	35.13787, -83.32031	37	RMA	36.45663, -83.58398			
9	CDA	35.39209, -79.47472	38	RMA	36.43896, -83.12255			
10	CDA	35.81812, -79.62896	39	RMA	36.22655, -88.33007			
11	CDA	35.4436, -79.98083	40	RMA	36.03133, -88.50585			
12	CDA	36.5626, -87.86865	41	RMA	36.10237, -89.40673			
13	CDA	36.36822, -88.72558	42	RMA	35.78217, -85.60546			
14	CDA	36.06686, -89.12109	43	RMA	35.78217, -86.06689			
15	CDA	36.45663, -83.58398	44	RMA	35.5501, -86.26464			
16	CDA	36.42128, -84.50683	45	RMA	35.62158, -85.71533			
17	CDA	35.38904, -89.01123	46	RMA	35.46066, -87.07763			
18	CDA	35.26356, -89.29687	47	RMA	33.6512, -91.75781			
19	RMA	36.19109, -93.66943	48	RMA	34.12544, -91.23046			
20	RMA	36.01356, -94.02099	49	RMA	38.85682, -78.44238			
21	RMA	35.94243, -93.58154	50	RMA	38.73694, -78.5083			
22	RMA	35.72867, -93.9331	51	RMA	38.65119, -78.6621			
23	RMA	35.94243, -93.03222	52	RMA	38.41055, -78.77197			
24	RMA	35.72867, -93.9331	53	RMA	38.46219, -78.2666			
25	RMA	35.67514, -93.49365	54	RMA	37.8575, -79.05761			
26	RMA	36.29741, -94.43847	55	RMA	37.68382, -78.88183			
27	RMA	35.65729, -92.79052	56	RMA	38.09998, -78.3325			
28	RMA	35.69299, -81.65039	57	RMA	38.15183, -79.76074			
29	RMA	35.64123, -82.15513	58	RMA	37.71859, -79.05761			

**Compiled patient data from Platts-Mills et al. (2013) and Chung et al. (2008)**

**CDA**=Alpha Gal allergy associated with clinical trial of the cancer drug Cetuximab.

**RMA**=Alpha Gal allergy associated with red meat allergy.

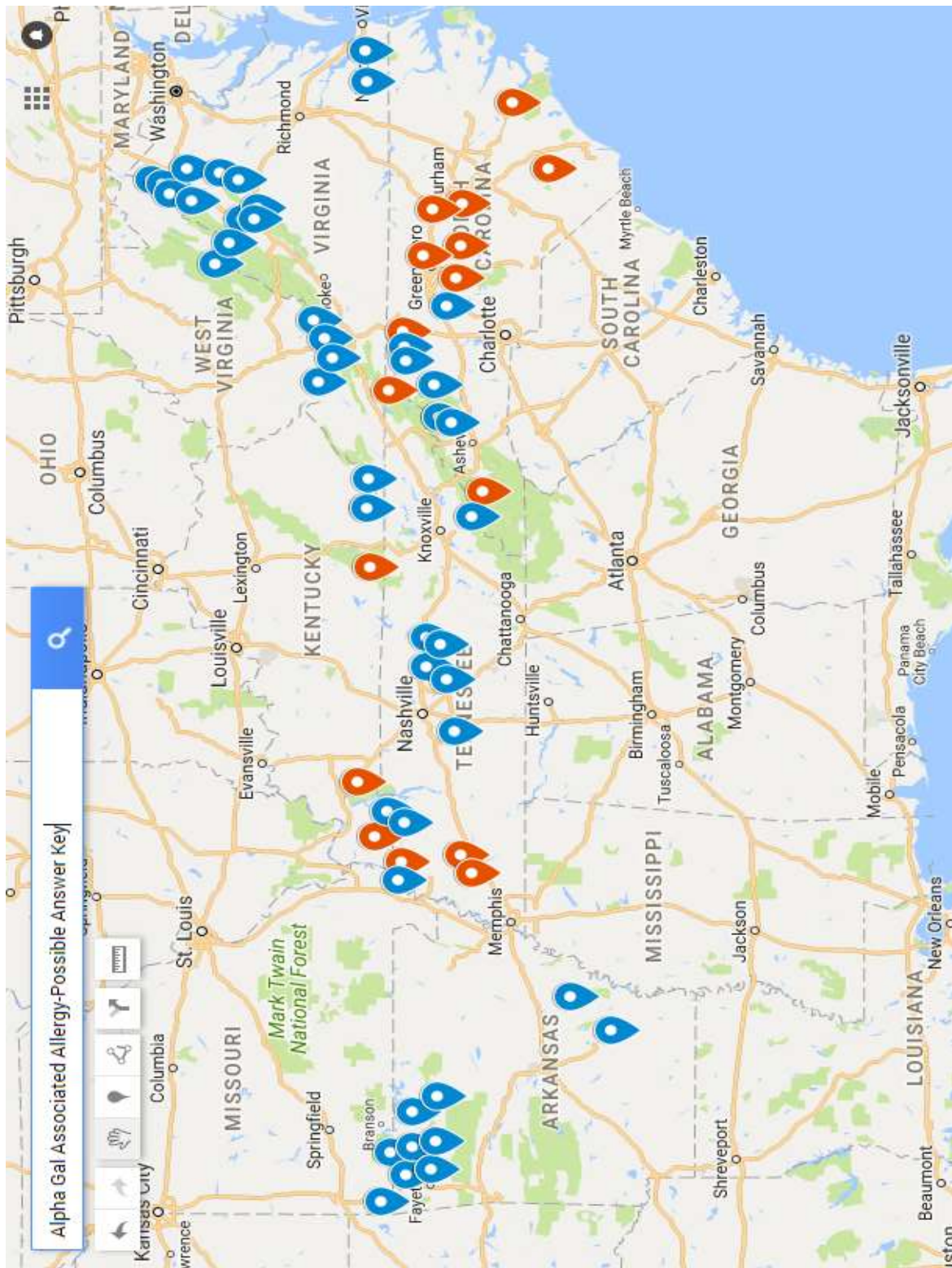
<https://drive.google.com/open?id=1NY00Y2BIulEQeg7hYudRI-QclfFhptBj&usp=sharing>

**Data Table for pen/paper collaborative version**

**Compiled patient data from Platts-Mills et al. (2013) and Chung et al. (2008)**

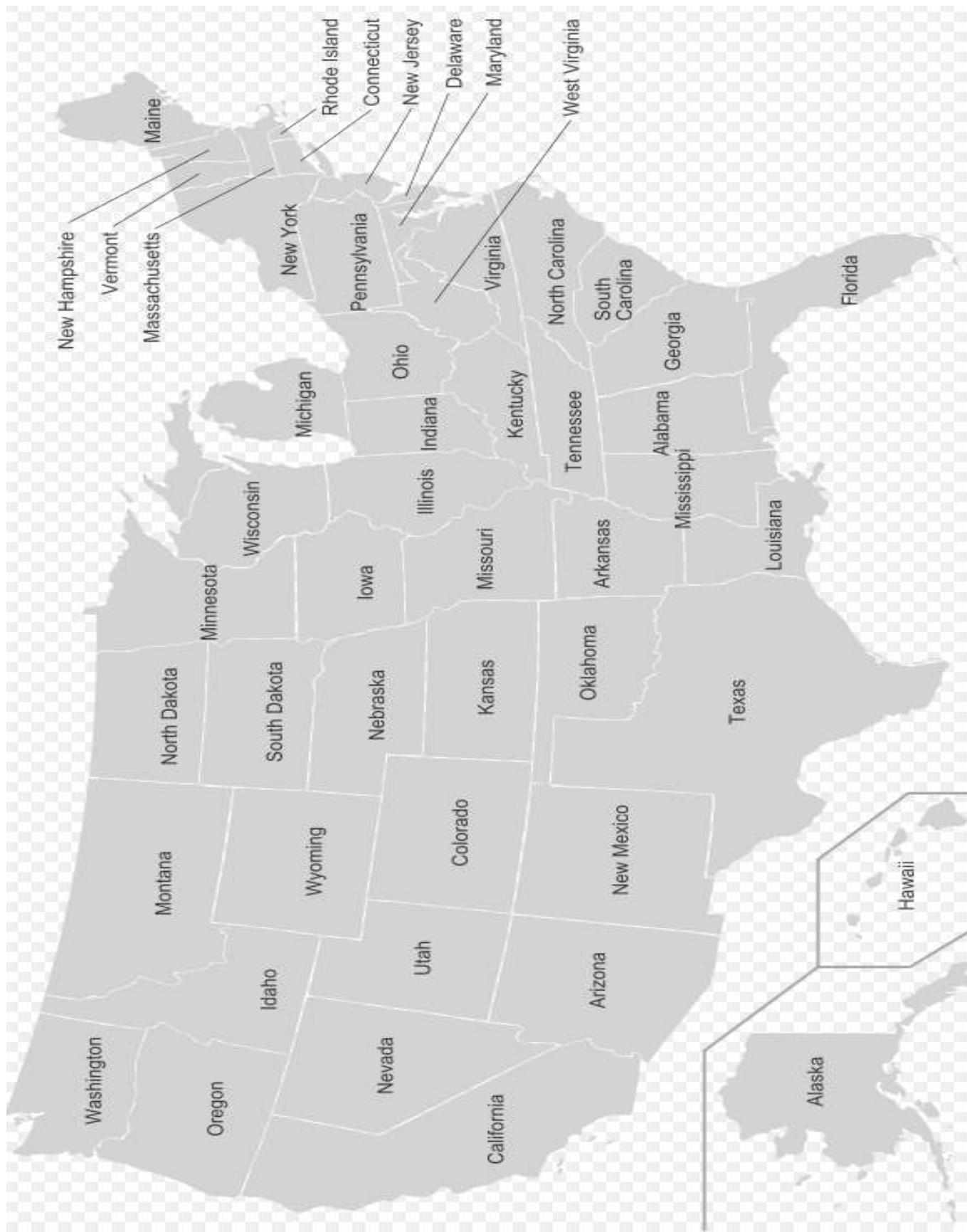
<b>Year Reported</b>	<b># of Cases</b>	<b>Geographic Region</b>	<b>Diagnosed Cause</b>
2007	4	Northeastern N. Carolina	Cetuximab allergy
2007	2	Northwestern N. Carolina	Cetuximab allergy
2007	1	Southeastern N. Carolina	Cetuximab allergy
2007	1	Southwestern N. Carolina	Cetuximab allergy
2007	3	Central N. Carolina	Cetuximab allergy
2007	2	Northeastern Tennessee	Cetuximab allergy
2009	3	Northwestern Tennessee	Cetuximab allergy
2009	2	Southeastern Tennessee	Cetuximab allergy
2009	9	Northwest Arkansas	Red meat allergy
2009	1	Northeastern N. Carolina	Red meat allergy
2009	2	Southeastern N. Carolina	Red meat allergy
2011	1	Southwestern N. Carolina	Red meat allergy
2011	6	Central N. Carolina	Red meat allergy
2011	2	Northeastern Tennessee	Red meat allergy
2011	3	Northwestern Tennessee	Red meat allergy
2011	2	Southeastern Tennessee	Red meat allergy
2011	5	Central Tennessee	Red meat allergy
2013	2	Southeastern Arkansas	Red meat allergy
2013	5	North Virginia	Red meat allergy
2013	7	Central Virginia	Red meat allergy
2013	4	Southwestern Virginia	Red meat allergy

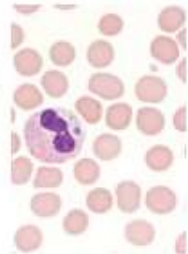
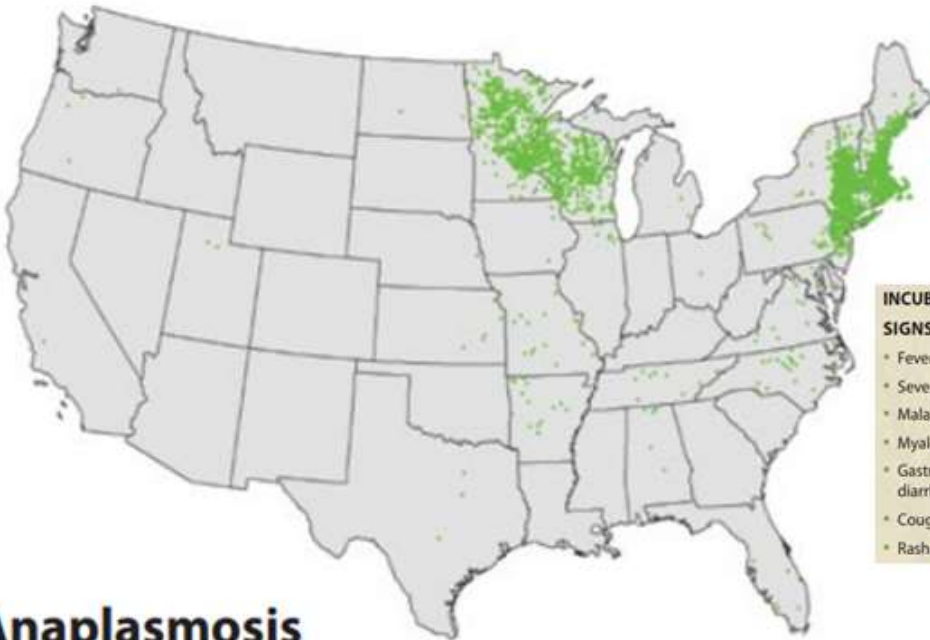




*After completing the initial layer of the medical geographers model, he recognizes a potential pattern in the distribution. A colleague of the department of medical geography works in in the entomology (study insects) department studying Ticks. Ticks are not technically insects they are more closely related to spiders and scorpions, but what catches the medical geographers attention is that a lot of the occurrences of the Alpha Gal allergy (both drug and red meat related) seem to be in what is considered Tick County. Tick's are known vectors for a variety of bacterial and viral diseases like lyme disease and rocky mountain spotted fever. The medical geographer calls over and has the tick specialist bring over their maps of tick borne diseases to see if their is any relationship. This would be the equivalent of adding an additional layer of data to the current GIS map created by the Alpha Gal allergy data.*

1. The maps provided indicate the occurrences of diseases associated with ticks in the United States. Analyze all of the new maps you have received and compare them to the map you have created illustrating the Alpha Gal allergy.
2. Eliminate the maps that you believe do not have a significant overlap in occurrences when looking at the Alpha Gal allergy and a specific tick borne disease.
3. After you have narrowed down your possible tick borne pathogens to two potential comparable distributions, review the background information for each pathogen to identify the tick that may be associated with the Alpha Gal allergy.





**INCUBATION PERIOD:** 1–2 weeks

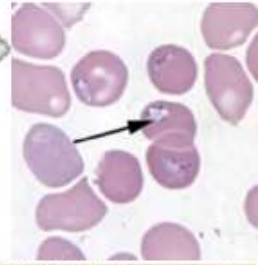
**SIGNS AND SYMPTOMS**

- Fever, shaking, chills
- Severe headache
- Malaise
- Myalgia
- Gastrointestinal symptoms (nausea, vomiting, diarrhea, anorexia)
- Cough
- Rash (rare cases)



**BLACKLEGGED TICK**  
*Ixodes scapularis*

# Anaplasmosis



**INCUBATION PERIOD:** 1–9+ weeks

**SIGNS AND SYMPTOMS**

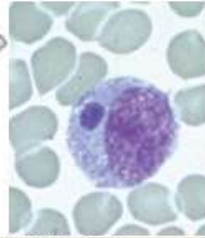
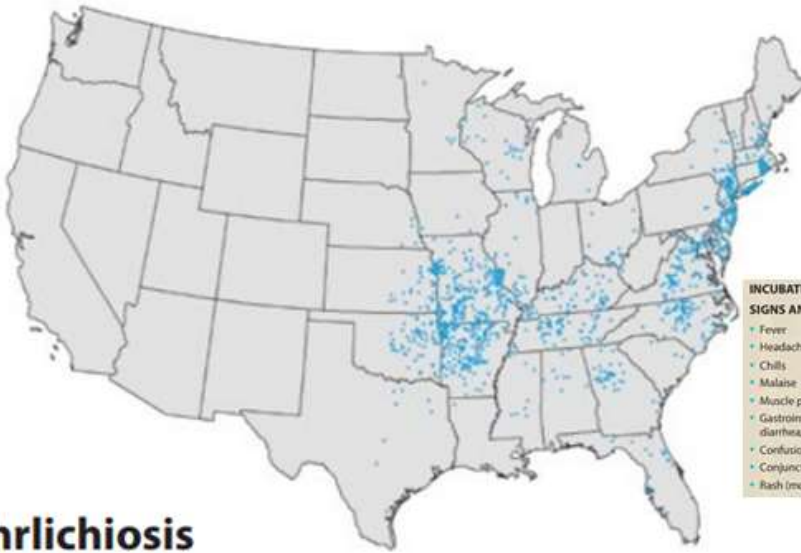
- Fever, chills, sweats
- Malaise, fatigue
- Myalgia, arthralgia, headache
- Gastrointestinal symptoms, such as anorexia and nausea (less common: abdominal pain, vomiting)
- Dark urine



**BLACKLEGGED TICK**  
*Ixodes scapularis*

# Babesiosis





**INCUBATION PERIOD:** 1–2 weeks

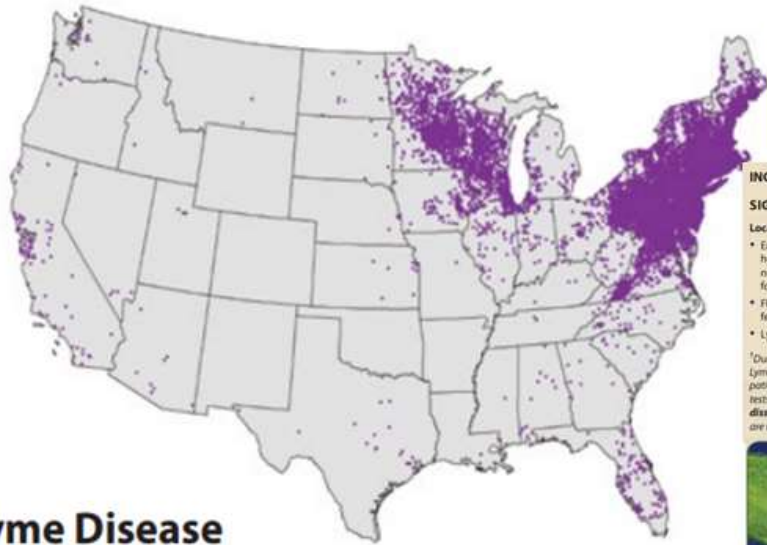
**SIGNS AND SYMPTOMS**

- Fever
- Headache
- Chills
- Malaise
- Muscle pain
- Gastrointestinal symptoms (nausea, vomiting, diarrhea, anorexia)
- Confusion
- Conjunctival injection
- Rash (more commonly reported among children)



**LONE STAR TICK**  
*Amblyomma americanum*

## Ehrlichiosis



**INCUBATION PERIOD:** 3–30 days

**SIGNS AND SYMPTOMS**

**Localized Stage<sup>1</sup>**

- Erythema migrans (EM)—red ring-like or homogenous expanding rash; classic rash not present in all cases. See examples on following pages.
- Flu-like symptoms – malaise, headache, fever, myalgia, arthralgia
- Lymphadenopathy

<sup>1</sup>During the **localized** (early) stage of illness, Lyme disease may be diagnosed clinically in patients who present with an EM rash. Serologic tests may be insensitive at this stage. During **disseminated** disease, however, serologic tests are usually positive.

**Disseminated Stage**

- Multiple secondary annular rashes
- Flu-like symptoms
- Lymphadenopathy
- Rheumatologic Manifestations**
- Transient, migratory arthritis and effusion in one or multiple joints
- Migratory pain in tendons, bursae, muscle, and bones
- Baker's cyst
- If untreated, arthritis may recur in same or different joints
- Cardiac Manifestations**
- Conduction abnormalities, e.g. atrio-ventricular node block
- Myocarditis, pericarditis



**BLACKLEGGED TICK**  
*Ixodes scapularis*

## Lyme Disease



## Tularemia



**INCUBATION PERIOD:** 3–5 days

### SIGNS AND SYMPTOMS

- Fever, chills
- Headache
- Malaise, fatigue
- Anorexia
- Myalgia
- Chest discomfort, cough
- Sore throat
- Vomiting, diarrhea
- Abdominal pain

*(Ulcer) Glandular*

- Localized lymphadenopathy
- Cutaneous ulcer at infection site (not always present)



**AMERICAN DOG TICK**  
*Dermacentor variabilis*



## Rocky Mountain Spotted Fever



**INCUBATION PERIOD:** 2–14 days

### SIGNS AND SYMPTOMS

- Fever, chills
- Severe headache
- Malaise
- Myalgia
- Gastrointestinal symptoms (nausea, vomiting, anorexia, abdominal pain, diarrhea, abdominal tenderness)
- Photophobia
- Focal neurologic deficits, including cranial or peripheral motor nerve paralysis or sudden transient deafness

### Maculopapular Rash

- Typically appears 2–5 days after the onset of fever
- Small, flat, pink, non-itchy spots (macules) initially appear on the wrists, forearms, and ankles then spread to the trunk and sometimes palms and soles.
- Rash may not develop until late in the disease process, after treatment should have already begun. Approximately 10% of RMSF patients never develop a rash at all.



**AMERICAN DOG TICK**  
*Dermacentor variabilis*

*Suspecting the Lone Star Tick (*Amblyomma americanum*) as the potential culprit that could have generated the allergic reaction that was recently reported, the medical geography reaches back out to the hospital. While speaking to the physician, the medical geographer suggests collecting a more detailed recent travel history, and finding out whether the family might have any pets that could be potential transporters of ticks into the home.*

*The physician happy to have a potential lead as to what is causing his patient's suspected allergy to meat follows up with a more detailed travel history where they learn of the families recent travel to North Florida and their outdoor activity. They also learn that the family does have a dog, who also accompanied them on their recent trip. The physician suggest the family take the dog to their vet to have it inspected for ticks.*

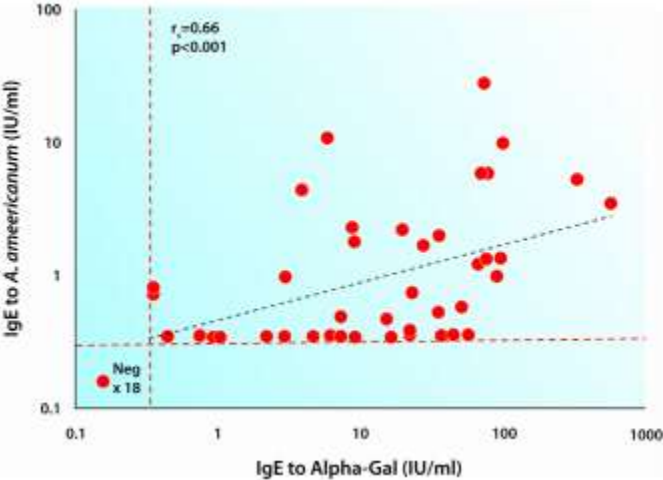
*Greg (Julie's husband) heeds the physician's advice, eager to get to the bottom of his wife's mysterious allergy. The trip to the vet proves fruitful if not, also disturbing. Using a tick brush, the vet located and preserved several different life stages of ticks extracted from Molly's coat and skin.*

1. Use the preserved samples or documented photographs and the resources provided here to identify or confirm the suspected tick species that may be responsible for the onset of the meat allergy.



[http://entnemdept.ufl.edu/creatures/urban/medical/lone\\_star\\_tick.htm](http://entnemdept.ufl.edu/creatures/urban/medical/lone_star_tick.htm)

<https://www.cdc.gov/lyme/resources/TickborneDiseases.pdf>





### **LESSON 3: Part 2 GIS Modelling-Making Connections**

**KEY QUESTION(S):** How do GIS modelers use abiotic and biotic data to determine the geographical distribution of organisms like the Lone Star Tick (*Amblyomma americanum*)?

**OVERALL TIME ESTIMATE:** 45 minutes

**LEARNING STYLES:** Visual, verbal

**VOCABULARY:**

**LESSON SUMMARY:** Now that students have a decent understanding of the relationship between the alpha gal allergy and the Lone Star tick as the vector for that allergy, they are prepared to look at the way that different environmental variables influence the distribution of organisms such as the Lone Star tick. In this lesson students will be provided with the output of a GIS modelling program called “Maxent”. The program analyzes actual observations of a target species and overlays it with land data including climate variables, foliage conditions, elevation, soil type etc. available through databases such as worldclim.org in order to predict the most accurate distribution of an organism across a larger geographical area. The goal for the students in this lesson will be to interpret some of the data provided by a maxent analysis of Lone Star Tick data in the state of Florida in order to generate plausible questions, generate hypotheses, and then write appropriate “model statements” supported by data generated by the maxent program.

#### **STUDENT LEARNING OBJECTIVES:**

The student will be able to...

- Analyze GIS maps of tick distribution in the state of Florida in order to ask testable questions about why tick populations are more abundant in some areas but less abundant in other areas.
- Generate hypothesis statements that might answer the questions students generated after analyzing the tick distribution data provided.
- Using the output of the maxent GIS program students can develop appropriate model statements explaining the relationships between specific environmental data and the distribution of tick species in the state of Florida.

#### **STANDARDS:**

##### **AP Biology Curriculum Standards**

**Science Practice 1:** The student can use representations and models to communicate scientific phenomena and solve scientific problems

**Essential knowledge 2.D.1:** All biological systems from cells and organisms to populations, communities and ecosystems are affected by complex biotic and abiotic interactions involving exchange of matter and free energy.

The stability of populations, communities and ecosystems is affected by interactions with biotic and abiotic factors. [See also 4.A.5, 4.A.6]

## Next Generation Sunshine State Standards

**SC.912.N.1.1** Define a problem based on a specific body of knowledge, for example: biology, chemistry, physics, and Earth/space science

**SC.912.L.17.2** Explain the general distribution of life in aquatic systems as a function of chemistry, geography, light, depth, salinity, and temperature

### **MATERIALS:**

Color copies of GIS Tick Distribution (laminated for extended use)

Copies of Maxent analysis results (laminated for extended use)

Copies of Student Guided Question Sheet

Chart Paper or Smartboard to collect student questions and hypotheses.

Optional Copy of “Ghost Moose Article-National Geographic” for optional extension

### **Background Information:**

The maxent program is an open source simple GIS program that can be downloaded and used by anyone to analyze species distribution data. For the purpose of this lesson the analysis has been completed for the students and the output is provided for them to interpret. The goal is to encourage students to ask appropriate questions, generate plausible hypotheses and then use available data to make appropriate “model statements” about relationships between environmental and abiotic data. The maxent analysis provided is a modified data set from the University of Florida’s Medical Geography department. Because of the subtle changes in environmental factors in Florida’s climate, certain variables have been exaggerated slightly to allow students the opportunity to identify appropriate model statements without having to overanalyze the data provided.

### **Advanced Preparation:**

Make enough color copies of GIS Tick Distribution for students to work in pairs (laminated for extended use)

Make enough color copies of Maxent analysis results for students to work in pairs (laminated for extended use)

Make enough copies of Student Guided Question Sheet so each student can report their findings

Make enough copies of “Ghost Moose Article-National Geographic” if choosing the optional extension activity.

### **Procedure and Discussion with Time Estimates**

#### Part 2

1. (5 minutes) Handout color copies of Lone Star Tick GIS distribution data. Ask the students to think back about where our patient Julie travelled for her camping trip. Does the distribution of the tick data presented on the GIS map support the possibility that the Lone Star tick could be responsible for generating the red meat allergy that she has recently experienced? Wait and troubleshoot any issues students are having with understanding the provided GIS maps.
2. (5 minutes) Have students work with their partners to review the GIS maps and then develop 2-3 testable questions regarding the data presented by the Lone Star Tick distribution maps.

Students will record their questions on the student handout provided to them. The teacher should move through the room listening for discussion and interjecting with feedback regarding the students' questions and their ability to be tested.

3. (5 minutes) Open the class up for discussion to collect potential questions students have generated from the analyzing the data. Give students the option to refine questions, or if they wish to pursue a new question that was presented by the group they should feel free to do so. If class discussion is limited, some sample questions are provided that are correlated to the maxent analysis in the later part of the lesson:

- a. Why are Lone Star ticks more abundant in the North part of the state than the south part of the state?
- b. Do Lone Star ticks prefer colder climates since they are more abundant in the northern part of the state?
- c. Does the amount of precipitation that the area receives influence the Lone Star ticks ability to survive?
- d. Do Lone Star ticks dislike coastal zones because it is more humid?

4. (5 minutes) Have students try to generate plausible testable hypothesis statements that would answer 2-3 questions (either the ones they generated or ones generated as part of the class discussion)

5. (20 minutes) Tell students that a model statement is a simplified statement based on a collection of data, whereby you can predict the outcome of one scenario based on the behavior of some extraneous factor. For example, a model statement might be: **As distance between spawning corals decreases, successful fertilization increases, and genetic diversity decreases.** Students will fill in the model statements on their student handouts to accurately represent the relationship between an environmental data and the distribution of the Lone Star Tick in Florida.

***Teaching Tip:** The maxent program can be downloaded for **free at....** if the teacher or the students are technological savy and the student computers have the ability to run Java they can download the tutorial data set and run through the actual tutorial to increase their understanding of how the GIS modeling program works.*

**Assessment Suggestions:**

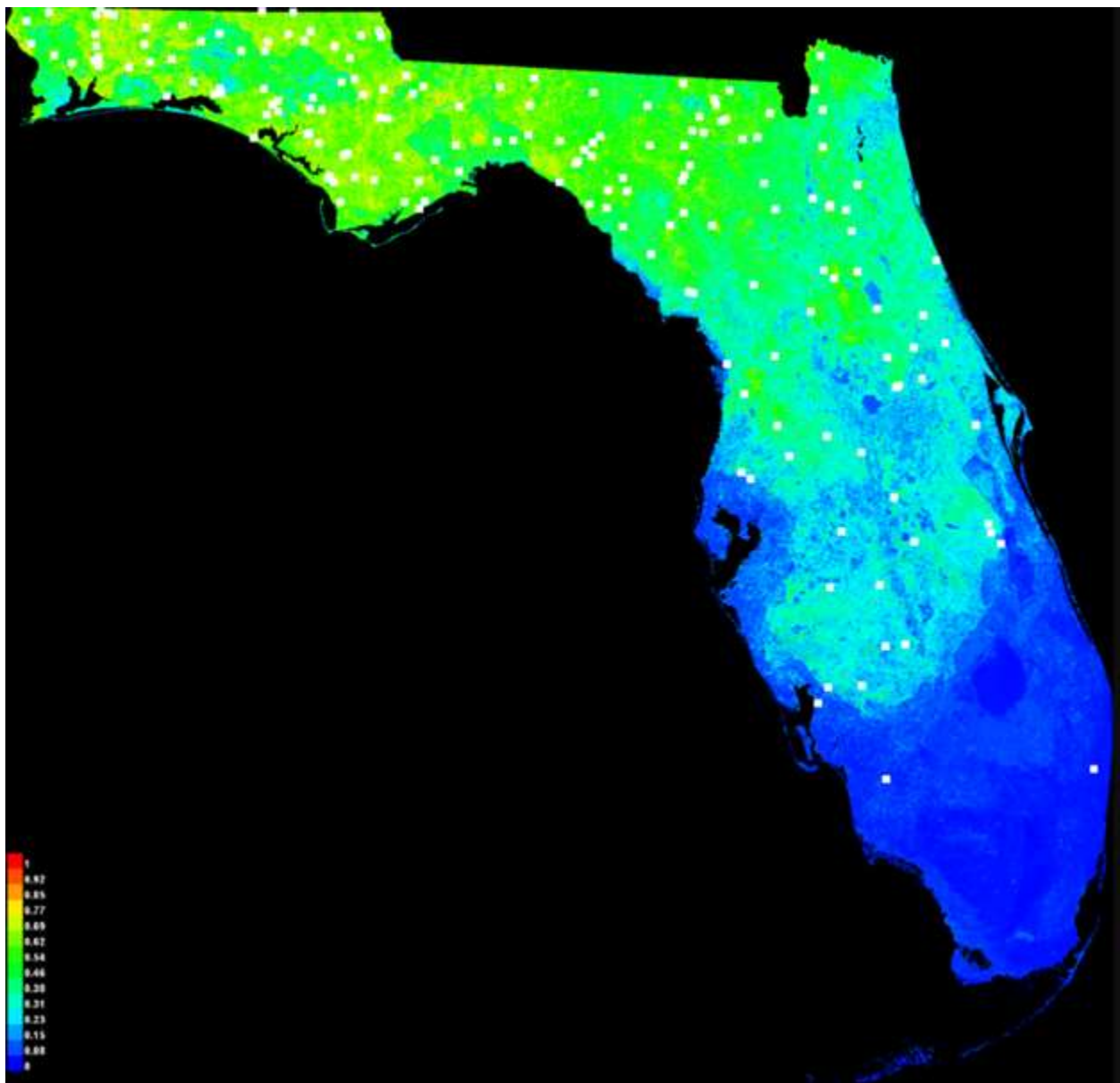
- Formative assessment based on student/class discussion
- Summative assessment collection of student handouts, the question/hypothesis statements could be graded for completion and the model statements could be graded for accuracy.

**Resources:**

**Insert Maxent website tutorial link here....**

### Lesson 3-Part 2: GIS Connecting the Dots

In this activity will take your GIS understanding a step further by using actual data of the Lone Star Tick's distribution in Florida and will use it to generate answerable scientific questions and plausible testable hypotheses. You will be provided with the results of a GIS data analysis where environmental data has been compared to actual tick distributions in order to generate model statements, whereby you can use environmental data to predict the distribution of the Lone Star tick and by doing so potentially identify areas where people may be susceptible to the Alpha Gal food allergy.

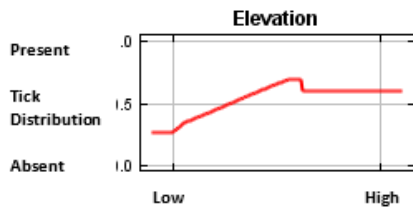


### GIS Connecting the Dots Student Handout

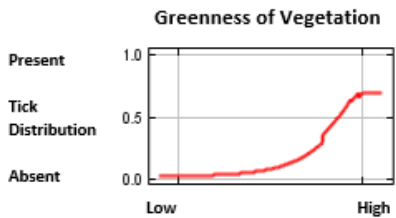
**Part 1**-The first part of this activity is very open ended. Since you will be generating the questions and subsequent hypotheses on your own there is technically no right or wrong answer, instead try to focus on the quality of the questions you develop and the testability of the hypotheses that you generate.

1. Thinking back to the background of our patient Julie and her family does the GIS map you have been provided with support or refute the possibility that the Lone Star tick (*Amblyomma americanum*) could be the cause of the recent onset of Julie's red meat allergy? Please justify your answer with support from the GIS map provide as well as your experiences over the last few days.
2. Analyze the GIS map provided and work with a partner to develop 2-3 relevant, testable questions regarding the distribution of the Lone Star tick (*Amblyomma americanum*)
3. After generating your questions, or feel free to use those generated in our discussion, write a testable hypothesis **and** null hypothesis statement for at least 2 of the questions from above.

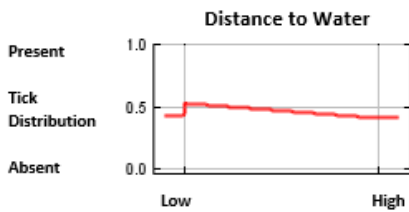
**Part 2-**The GIS maps you reviewed in part 1 of this activity is based on actual observations of the Lone Star tick (*Amblyomma americanum*). The GIS software analyzes where ticks are found and overlays it with the environmental conditions present there, the program then looks for other parts of the state with similar values of supporting variables in order to predict where the Lone Star tick might be found across the state. The more data that is included in the model the more likely the model will make accurate predictions about the intended outcome. Below is a collection of the data used to generate the GIS model you used in part 1, you are responsible for analyzing the data and writing a brief model statement based on the environmental factor and its role in shaping tick distribution. The first one has been done for you.



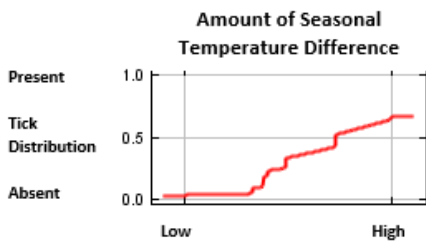
As elevation increases the likelihood of ticks being present increases until an optimum elevation is reached.



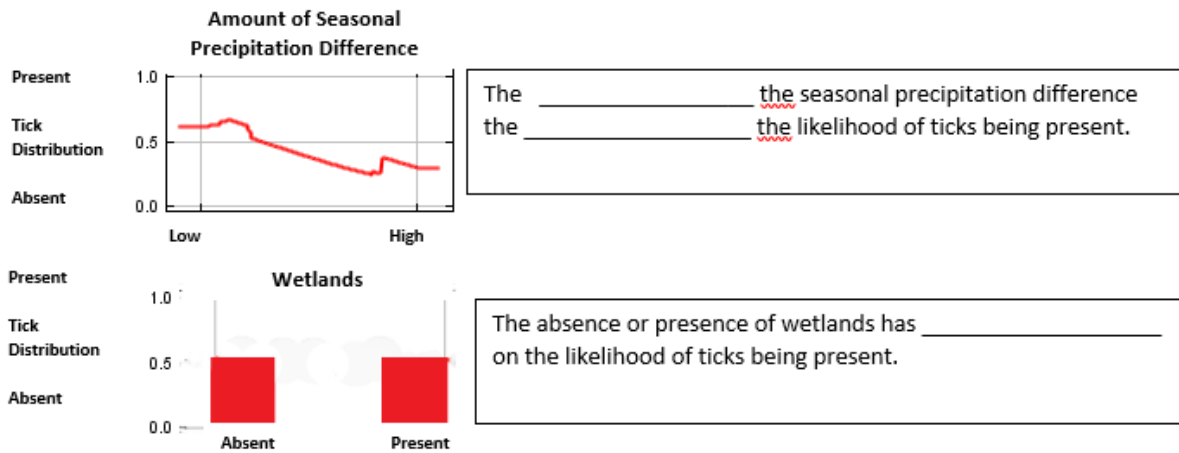
As the greenness of the vegetation \_\_\_\_\_ the likelihood of ticks being present \_\_\_\_\_.



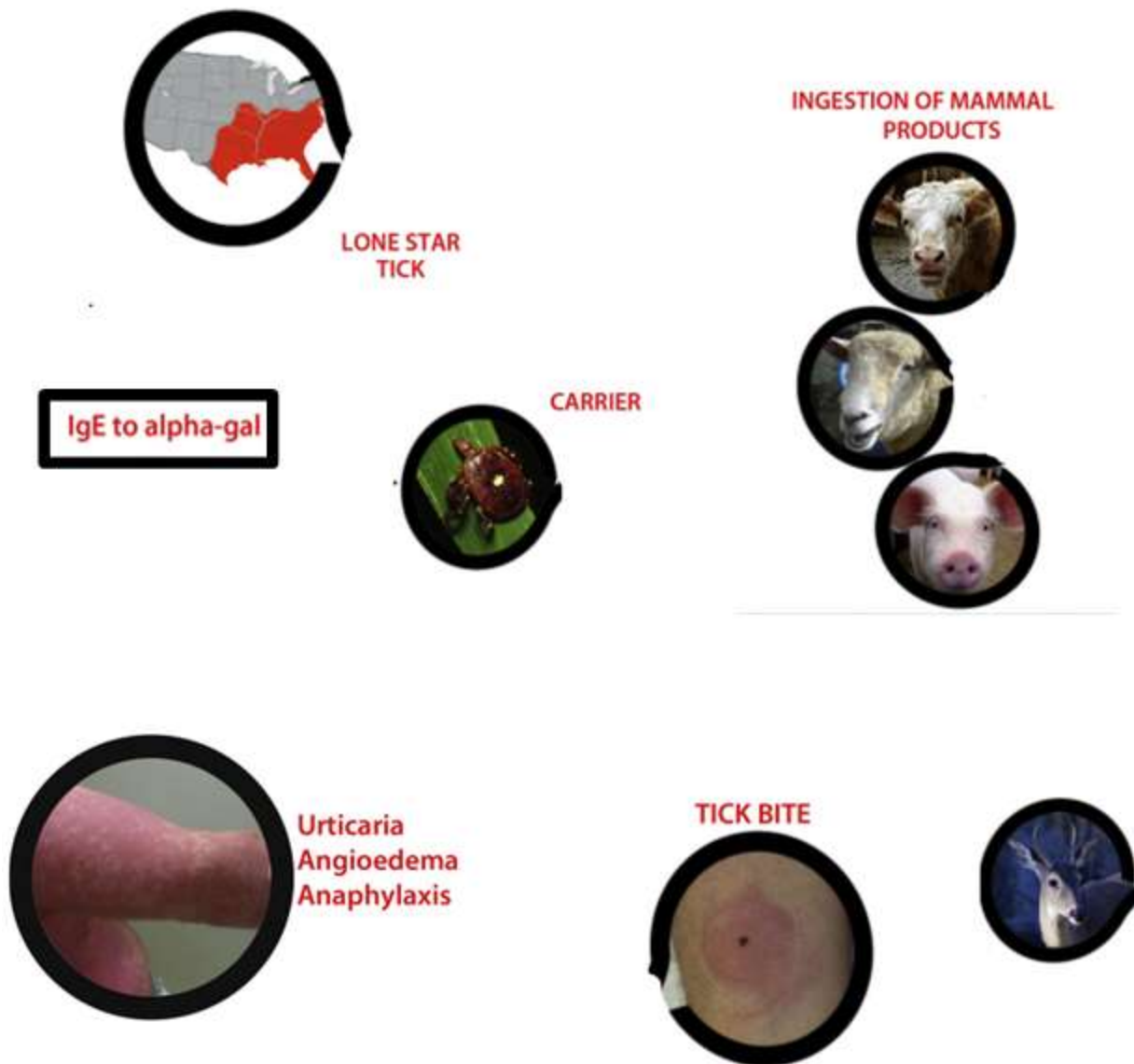
As the distance to water \_\_\_\_\_ the likelihood of ticks being present \_\_\_\_\_.



The \_\_\_\_\_ the seasonal temperature difference the \_\_\_\_\_ the likelihood of ticks being present.



Some models may be used to connect organism and their relationships with each other in order to explain the cause and effect between events. Draw arrows to connect the events as you understand them thus far that lead to the eventual Alpha Gal meat allergy.



4. Optional Application: Review the article at the following link.  
<https://news.nationalgeographic.com/2015/06/150601-ghost-moose-animals-science-new-england-environment/> While you are reading the article think about how GIS modelling could be utilized in the scenario presented in the article.



## Lesson 4

### Lesson 4: Bringing it all together-The alpha-gal story: Lessons learned from connecting the dots

**KEY QUESTION(S):** How are the conclusions drawn from your experience so far linked to the real world efforts of scientists and researchers?

**OVERALL TIME ESTIMATE:** 45 minutes

**LEARNING STYLES:** Verbal

#### **VOCABULARY:**

**LESSON SUMMARY:** This lesson is presented here as the summary lesson for the Alpha-Gal allergy case study we have been exploring. In this lesson students will review an article from a scholarly journal that illustrates the efforts of the many different scientists and researchers in attempting to uncover the cause and effect of the onset of the red meat allergy. Students will evaluate the article together (in small groups or as an entire class) and answer guided questions that will determine their overall understanding of the relationship between the immune response and ticks as a vector for the spread of this red meat allergy.

#### **STUDENT LEARNING OBJECTIVES:**

The student will be able to...

- Read a scientific article and engage in in depth discussion regarding the content of the article.
- Answer guided questions about a journal published in a scientific journal.
- Relate the relationship between different categories of macromolecules, the human immune response, tick distribution and the overall red meat allergy.

#### **STANDARDS:**

##### **AP Biology Curriculum Standards**

**Essential knowledge 2.D.4: Plants and animals have a variety of chemical defenses against infections that affect dynamic homeostasis**

**(B)** Mammals use specific immune responses triggered by natural or artificial agents that disrupt dynamic homeostasis.

**Science Practice 1:** The student can use representations and models to communicate scientific phenomena and solve scientific problems

**Science Practice 5:** The student can perform data analysis and evaluation of evidence

##### **Next Generation Sunshine State Standards**

**SC.912.L.14.52** Explain the basic functions of the human immune system, including specific and nonspecific immune response, vaccines, and antibiotics.

**SC.912.L.14.6** Explain the significance of genetic factors, environmental factors, and pathogenic agents to health from the perspectives of both individual and public health.

**SC.912.L.18.1** Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules.

**SC.912.N.1.1** Define a problem based on a specific body of knowledge, for example: biology, chemistry, physics, and Earth/space science

**MATERIALS:**

Copies of the the article:**The alpha-gal story: Lessons learned from connecting the dots**

Highlighters, colored pens or pencils to mark the text.

Copies of the guided question hand out for students to complete their answers.

**Background Information:**

**Advanced Preparation:**

**Procedure and Discussion with Time Estimates**

***Teaching Tip:***

**Assessment Suggestions:**

**Resources:**

# The alpha-gal story: Lessons learned from connecting the dots

John W. Steinke, PhD, Thomas A. E. Platts-Mills, MD, PhD, FRS, and Scott P. Commins, MD, PhD *Charlottesville, Va*

## INFORMATION FOR CATEGORY 1 CME CREDIT

Credit can now be obtained, free for a limited time, by reading the review articles in this issue. Please note the following instructions.

**Method of Physician Participation in Learning Process:** The core material for these activities can be read in this issue of the Journal or online at the JACI Web site: [www.jacionline.org](http://www.jacionline.org). The accompanying tests may only be submitted online at [www.jacionline.org](http://www.jacionline.org). Fax or other copies will not be accepted.

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**Overall Purpose/Goal:** To provide excellent reviews on key aspects of allergic disease to those who research, treat, or manage allergic disease.

**Target Audience:** Physicians and researchers within the field of allergic disease.

**Accreditation/Provider Statements and Credit Designation:** The American Academy of Allergy, Asthma & Immunology (AAAAI) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians. The AAAAI designates this journal-based CME activity for a maximum of 1 AMA PRA Category 1 Credit™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

**List of Design Committee Members:** John W. Steinke, PhD, Thomas A. E. Platts-Mills, MD, PhD, FRS, and Scott P. Commins, MD, PhD

**Disclosure of Significant Relationships with Relevant Commercial Companies/Organizations:** J. W. Steinke has received research support from Genentech (HSR 17123), has received a lecture honorarium from Northwestern, and receives royalties for human mast cell line developed. T. A. E. Platts-Mills has received research support from the National Institutes of Health (NIH; AI-20565)/National Institute of Allergy and Infectious Diseases (NIAID; AI-085190), has received travel support from Phadia/Thermo Fisher, and receives royalties from IBT/Viracor. S. P. Commins has received research support from the NIH/NIAID (AI-20565; AI-085190; grant K08), has received consultancy fees from Sanofi, and receives royalties from UpToDate.

### Activity Objectives

1. To describe the history and presenting symptoms of galactose-alpha-1,3-galactose (alpha-gal).
2. To describe the pathophysiology of the IgE-mediated response to alpha-gal in human subjects.
3. To list the diagnostic approach to alpha-gal.

**Recognition of Commercial Support:** This CME activity has not received external commercial support.

**List of CME Exam Authors:** Jill Hanson, MD, Alison Humphrey, MD, David Jara, MD, Neha Patel, MD, and Paul Dowling, MD

**Disclosure of Significant Relationships with Relevant Commercial Companies/Organizations:** The exam authors disclosed no relevant financial relationships.

Anaphylaxis is a severe allergic reaction that can be rapidly progressing and fatal, and therefore establishing its cause is pivotal to long-term risk management. Our recent work has identified a novel IgE antibody response to a mammalian oligosaccharide epitope, galactose-alpha-1,3-galactose (alpha-gal). IgE to alpha-gal has been associated with 2 distinct forms of anaphylaxis: (1) immediate-onset anaphylaxis during first exposure to intravenous cetuximab and (2) delayed-onset anaphylaxis 3 to 6 hours after ingestion of mammalian food products (eg, beef and pork). Results of our studies and those of others strongly suggest that tick bites are a cause, if not the only significant cause, of IgE antibody responses to alpha-gal in the southern, eastern, and central United States; Europe; Australia; and parts of Asia. Typical immune responses to carbohydrates

are considered to be T-cell independent, whereas IgE antibody production is thought to involve sequential class-switching that requires input from T cells. Therefore, establishing the mechanism of the specific IgE antibody response to alpha-gal will be an important aspect to address as this area of research continues. (*J Allergy Clin Immunol* 2015;135:589-96.)

**Key words:** Anaphylaxis, delayed reaction to red meat, galactose-alpha-1,3-galactose

Discuss this article on the JACI Journal Club blog: [www.jaci-online.blogspot.com](http://www.jaci-online.blogspot.com).

Hypersensitivity in the allergic setting refers to immune reactions stimulated by soluble antigens that can be rapidly progressing and, in the case of anaphylaxis, are occasionally fatal. Because the number of known exposures associated with anaphylaxis is limited, identification of novel causative agents is important in facilitating both education and other allergen-specific approaches that are crucial to long-term risk management. Within the last 10 years, several seemingly separate observations were recognized as related, all of which resulted from the development of antibodies to a carbohydrate moiety on proteins in which exposure differed from airborne allergens but

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<http://dx.doi.org/10.1016/j.jaci.2014.12.1947>



#### Abbreviations used

Alpha-gal: Galactose-alpha-1,3-galactose  
LDL: Low-density lipoprotein  
RMSF: Rocky Mountain spotted fever  
VLDL: Very low-density lipoprotein

that were nevertheless capable of producing anaphylactic and hypersensitivity reactions. Our recent work has identified these responses as being due to a novel IgE antibody directed against a mammalian oligosaccharide epitope, galactose-alpha-1,3-galactose (alpha-gal).<sup>1</sup> This review will present the history and biology of alpha-gal and discuss the evidence that the IgE response to alpha-gal is different from typical IgE responses directed toward protein allergens.

### CETUXIMAB-INDUCED HYPERSENSITIVITY REACTIONS

In 2004, ImClone and Bristol-Myers Squibb were investigating an mAb (cetuximab) specific for the epidermal growth factor receptor in clinical trials for the treatment of metastatic colorectal cancer. Early in those studies, it became clear that the antibody was causing hypersensitivity reactions; however, these reactions were occurring primarily in a group of southern US states (Table 1). These reactions to cetuximab developed rapidly, and symptoms often peaked within 20 minutes after or during the first infusion of the antibody and occasionally proved fatal.<sup>1-3</sup> Because of delays in marketing, it was not until 2006 that the true severity of the reactions became obvious.<sup>2</sup>

At this time, our group began preliminary experiments examining the IgE response to this molecule. Dr Hatley, who was working in Bentonville, Arkansas, convinced our group to develop a new version of the IgE fluorometric enzyme immunoassay or CAP assay to cetuximab using the streptavidin technique. In this assay streptavidin is coupled to the solid phase of the CAP to provide a matrix for the binding of biotinylated novel or purified allergens.<sup>3</sup>

We were subsequently asked to investigate the reactions to cetuximab, in part because we had already developed the IgE assay to cetuximab. In collaboration with Dr Chung from Nashville, Dr Mirakhor from Bristol-Myers Squibb, and Dr Hicklin from ImClone, we demonstrated that the patients who had reactions to cetuximab also had IgE antibodies specific for this molecule before they started treatment.<sup>1</sup> The question remained as to what epitope the IgE antibody was recognizing on the cetuximab molecule.

Early work by Karl Landsteiner discovered that all human subjects had antibodies to a blood group "B-like" oligosaccharide found on nonprimate red blood cells.<sup>5</sup> That antigen was subsequently identified as alpha-gal, which represents a major transplantation barrier between primates and other mammals.<sup>6-8</sup> Antibodies against alpha-gal are present in all nonimmunocompromised human subjects, and some early studies suggested that the IgG antibodies against alpha-gal constituted about 1% of circulating immunoglobulins in human subjects, apes, and Old World monkeys.<sup>9</sup> Recent work in our laboratory with specific assays for IgG antibodies suggests that the percentages are not this high. As discussed below, the fact that all nonprimate

mammals, including mice, can make oligosaccharides that are foreign to human subjects is an important component of our story.

### CARBOHYDRATE ANALYSIS OF CETUXIMAB

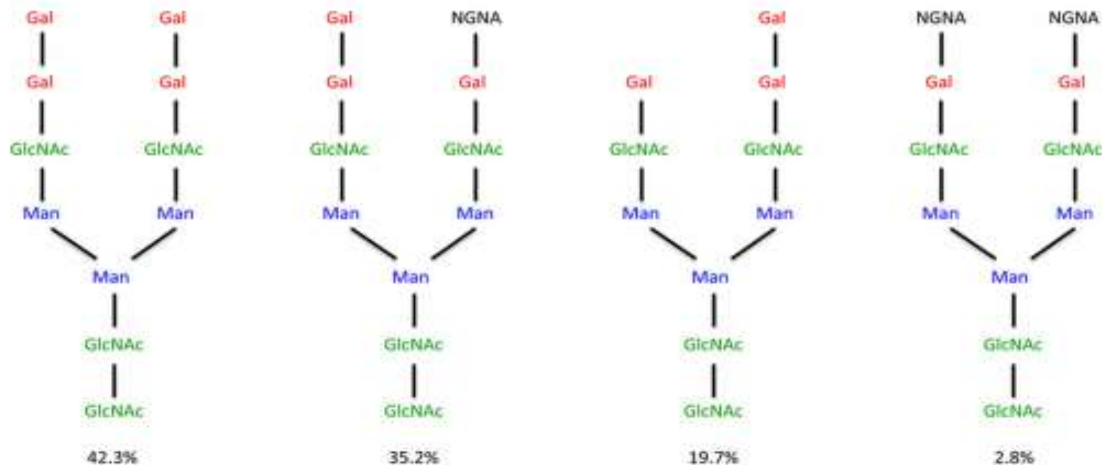
Glycosylation of proteins is a posttranscriptional modification that can play key roles in many processes, including protein folding, protein stability, intracellular trafficking, and cellular adhesion, as reviewed by Hurtado-Guerrero and Davies.<sup>10</sup> Characterization of cetuximab glycosylation, as measured based on peak area on time-of-flight mass spectrometric spectra, revealed 21 distinct oligosaccharide structures, of which approximately 30% have 1 or more alpha-1,3-linked galactosyl residues.<sup>11</sup> Analysis of the IgE antibodies to cetuximab demonstrated that these antibodies were specific for the oligosaccharide residues on the heavy chain of the Fab portion of the mAb. From the known glycosylation of the molecule at amino acids 88 and 299 (Fig 1),<sup>11</sup> alpha-gal was identified as the relevant epitope. Of the total alpha-gal in cetuximab, most of it is located in the Fab domain (Fab domain: 990 nmol alpha-gal/ $\mu$ mol IgG vs Fc domain: 140 nmol alpha-gal/ $\mu$ mol IgG).<sup>11</sup> Recent mass spectrometric analysis indicates that glycosylation of cetuximab might be more complex than previously thought, containing both diantermary and triantemary structures.<sup>12</sup> Synthesis of alpha-gal requires the gene encoding alpha-1,3-galactosyltransferase. In human subjects and higher primates this gene is not functional, and therefore these species cannot produce alpha-gal, which in turn makes it possible for these animals to initially make IgG and IgM antibodies directed toward this oligosaccharide.<sup>7,13</sup> How IgE to alpha-gal gets made and the nature of the IgE response will be considered later.

Of considerable importance to the development of biologics, in particular mAbs, is the observation that murine cell lines, such as NS0 and Sp2/0, can synthesize galactose in an alpha-1,3 linkage such that alpha-gal is present on the molecules. Sp2/0 was the cell line used to produce cetuximab. In those subjects with IgE to alpha-gal ( $\geq 0.35$  IU/mL), reactions are likely to occur directed against this mAb.<sup>3</sup>

### THE RED MEAT CONNECTION

During this same time period (2006-2008), we evaluated a number of patients, most of whom spent a significant amount of time outdoors, who had presented with episodes of generalized urticaria, angioedema, or recurrent anaphylaxis. The importance of the time spent outdoors was not clear at that time. There was no obvious immediate cause for the symptoms, but in several cases the patients reported that they believed the reactions might be due to consumption of meat 3 to 5 hours earlier. Skin prick tests were performed with commercial extracts of beef, pork, or lamb and produced small wheals only 2 to 4 mm in diameter that often would be interpreted as negative results. However, given the compelling history described by the patients, we extended our analysis to intradermal skin testing with commercial meat extracts or skin prick tests with fresh meat extracts, both of which demonstrated strong positive results.<sup>14</sup> These results were confirmed with blood tests for specific IgE antibody to red meats.<sup>14</sup>

Although not published, similar sensitivity to red meat had been previously noted in Georgia. Starting in 1989, Mrs Sandra Latimer, together with Dr Antony Deutsch from Athens, Georgia,



**FIG 1.** Carbohydrate structure of N-linked glycans on the Fab portion of cetuximab, as determined by using mass spectrometry. Alpha-gal (red), mannose (blue), N-acetylglucosamine (green), and N-glycolyl neuraminic acid (black) are shown. Percentages represent the proportion of the indicated structures found on the Fab region of cetuximab. Adapted from Qian et al.<sup>11</sup>

collected 10 cases of delayed reactions to mammalian meat and made a connection with the occurrence of tick bites several weeks or months before the first episode of hives or anaphylaxis. They presented these findings to the Georgia Allergy Society and to the US Centers for Disease Control and Prevention in 1991, but no additional reports or statements were issued by either of these organizations.

The characteristics of red meat allergy are different from typical allergic reactions. Common complaints include both gastrointestinal symptoms and urticaria, but unlike most allergic reactions, patients do not have any symptoms for at least 2 hours after eating red meat, whereas many reactions are delayed for 3 to 5 hours or even longer. Nonetheless, symptoms can be severe or even life-threatening. Many of the patients described nausea, diarrhea, or indigestion before a reaction; however, the most common symptom reported was itching. The presence of symptoms before a severe reaction is common but not a requirement. Many patients do not have any symptoms, and symptoms do not occur with every exposure to red meat even in those who have had them previously. All of the patients had consumed red meat without complications for many years before the onset of the syndrome. Although some patients had a prior history of allergy, most of them had no previous allergic symptoms, and thus an atopic disposition does not appear to predispose patients to this kind of IgE response.

Three observations led us to investigate whether IgE antibodies to alpha-gal were present in the sera of adult patients reporting reactions to beef. Alpha-gal is known to be present on both tissues and meat from nonprimate mammals,<sup>15</sup> the antibodies causing reactions to cetuximab were directed against alpha-gal, and the geographic distribution of the reactions to cetuximab overlapped the same geographic area where the red meat–induced reactions were occurring. Not surprisingly, the patients’ sera had positive results for IgE to beef, pork, lamb, cat, and dog but not to nonmammalian meat, such as turkey, fish, or chicken.<sup>14,16</sup> The

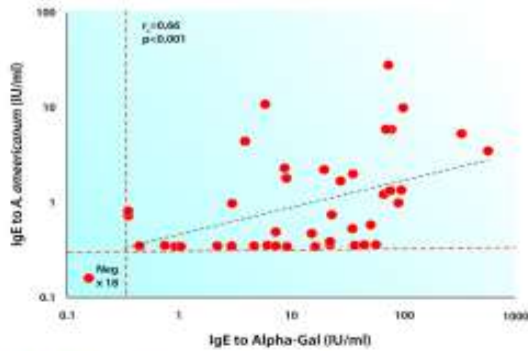
presence of alpha-gal was confirmed by using 2 different absorption assays, one with alpha-gal on human serum albumin and the other with mammalian or beef thyroglobulin, which is heavily decorated with alpha-gal. The glycosylated antigens were bound to sepharose beads.<sup>14</sup> In each case, levels of the specific IgE binding to beef, pork, lamb, cat, and dog were reduced by greater than 75%. More recently, evidence has been obtained from a study examining beef extracts using 2-dimensional gel electrophoresis. The authors demonstrated 7 alpha-gal-containing IgE-binding proteins, 4 of which survived heating the beef extract.<sup>17</sup>

How alpha-gal is structurally expressed on red meat remains unclear. Also unclear is whether differences in the structure exist and whether these differences affect IgE binding. The terminal carbohydrate residue on red meat is likely alpha-gal based on the binding of IgE from sera of patients with red meat allergy to cetuximab, which, as discussed, also has terminal alpha-gal residues. However, one can envision a difference in carbohydrate structure, such that only a single exposed alpha-gal-binding site is present in the oligosaccharide chain on meat, contrasting the 2 found predominately on cetuximab. The majority of alpha-gal found on cetuximab has a diantennary structure (Fig 1). The structure on meat has not been determined. Whether having 2 alpha-gal residues on the terminus of the carbohydrate structure has an effect on the strength of IgE binding is unknown.

#### ARE TICK BITES RESPONSIBLE FOR THE INDUCTION OF IgE ANTIBODY TO ALPHA-GAL?

In 2008, as the specificity of the IgE antibodies to alpha-gal that caused reactions to cetuximab became clearer, the number of reports describing delayed reactions to red meat was also increasing. A relationship between mammalian meat allergy and tick bites had already been suggested in Australia<sup>15</sup>; however, the role of alpha-gal was not known, and the tick connection was





**FIG 2.** Relationship of IgE to alpha-gal with IgE to *Amblyomma americanum* (lone star tick). Levels of specific IgE to alpha-gal and *A. americanum* were measured by using ImmunoCAP, and the correlation between the 2 types of IgE was determined by using Spearman correlation ( $r_s = 0.66$ ,  $P < .001$ ).

not yet obvious in the United States. What caught our attention was that both cetuximab reactions and delayed reactions to red meat were being reported from the same region of the country, a group of southeastern states. However, it was not clear why these cases were geographically localized, and the only area that was comparable was the maximum incidence of Rocky Mountain spotted fever (RMSF).

At this time, red meat allergy developed in 3 members of our group, and each one distinctly remembered being bitten by ticks weeks or months before the development of symptoms. Sera from these persons that had been obtained before the tick bite were compared with sera collected after the bite, and it was found that serum levels of IgE to alpha-gal had increased dramatically (4- to 10-fold).

Following up on this connection, we started to ask patients about tick bites and rapidly became aware that most of those with delayed anaphylaxis had experienced recent bites from adult or larval ticks. Examination of US Centers for Disease Control and Prevention maps of the distribution of the tick *Amblyomma americanum* (lone star tick) revealed an overlap with the region of both cetuximab sensitivity and red meat allergy. Additional indications that tick bites are involved in the development of specific IgE to alpha-gal include histories of bites that have itched for 2 or more weeks, a significant correlation between IgE antibodies to alpha-gal and IgE to lone star tick (Fig 2), and prospective data on the increase in IgE levels to alpha-gal after known lone star tick bites.<sup>19</sup> Allergy to red meat is now being reported in other countries, but the ticks giving rise to this response are not the same species as in the United States. In Europe *Ixodes ricinus* has been implicated, whereas in Australia the relevant tick is *Ixodes holocyclus*.<sup>18,20,21</sup> It appears that *Ixodes scapularis*, the main vector of Lyme disease (*Borrelia burgdorferi* infection) in the United States, does not induce IgE to alpha-gal, and unlike bites of the lone star tick, bites of *I. scapularis* that transmit Lyme disease are not associated with itching.<sup>22</sup>

Given that tick bites represent the most important cause of alpha-gal sensitization in the United States, Sydney, and

Stockholm, why has our recognition of this problem increased so dramatically over the past 10 years? The increase in lone star ticks parallels the increase in the deer population, a major carrier of these ticks, throughout the United States over the last 30 to 40 years,<sup>23,24</sup> making it more likely that persons who walk in the woods or in long grass will be bitten at some point. The increasing deer population can also be linked to the enactment of leash laws for dogs, a decrease in the number of hunters, and movement of deer into suburban areas. This last point is important because the deer provide a means for the ticks to be transported over large geographic areas quickly. Clearly, the increase in tick exposure is one plausible explanation for the increase in the number of cases. However, the data from different countries demonstrate that not all tick bites *per se* or tick bites from one particular species result in the problem (Table II).

The epidemiologic evidence in the United States would suggest that the increase in the deer population has played an important role. However, it is important to remember that there are at least 3 theories about how tick bites give rise to an IgE response:

1. the response is induced by the normal (ie, tick-derived) constituents of their saliva;
2. residual mammalian glycoproteins or glycolipids are present in the tick from a previous blood meal and they are responsible for inducing the response to alpha-gal; and
3. the response is induced by another organism that is present in the tick.

The best recognized organisms present as commensals on ticks are *Rickettsia* species, such as those that cause RMSF, or bacteria, such as *B. burgdorferi*, which is not found in the lone star tick (*A. americanum*). Other organisms are possible, but none have been recognized.

It might be thought that the IgE response seen with seed ticks would argue against either residual mammalian proteins or other organisms; however, transovarial transmission of RMSF is well recognized. Sorting out these possibilities is the subject of ongoing investigation.

#### A BROADER UNDERSTANDING OF ALPHA-GAL

The early reports of alpha-gal sensitivity were mostly from adults, with very few reports of affected children. However, children often have urticaria, angioedema, or recurrent anaphylaxis for which the cause is unknown. We identified 51 children aged 4 to 17 years with symptoms consistent with possible delayed allergic reactions to mammalian foods and measured IgE levels to alpha-gal in their sera. Serum IgE levels to alpha-gal were high in 45 of the subjects, and there was a strong correlation with beef IgE levels, as previously observed in adults.<sup>10</sup> When questioned, these children gave a history of symptoms 3 to 6 hours after ingestion of meat, and many could recall recent tick bites. The geographic distribution of affected children matches that of adults, namely the southeastern United States.

For protein allergens, there is a strong correlation between atopic sensitivity and asthma.<sup>25-27</sup> It is unknown whether this same relationship exists when an oligosaccharide is the target of the IgE response. Three populations were examined: one with high levels of IgE to alpha-gal and anaphylaxis, angioedema, or acute urticaria after ingestion of red meat; another of persons admitted to the emergency department for an acute asthma



**TABLE I.** Time course of the alpha-gal story

Year: Events leading to our understanding of red meat allergy
–2000: At least 2 groups reported cases of meat allergy that started after tick bites.
2003: IgE to cat allergens is common in an African village but not related to symptoms.
2005: There are reports of hypersensitivity reactions to first infusion of cetuximab in clinical trials.
2007: Severe reactions to cetuximab are common in Tennessee, North Carolina, Arkansas, Missouri, and Virginia.
2007: Two cases in Virginia of adult-onset delayed anaphylaxis occurring 3 to 6 hours after eating beef are reported.
2008: Alpha-gal is identified as the epitope on cetuximab.
2009: Twenty-four cases of delayed anaphylaxis to red meat are found in the United States. Multiple cases of meat allergy after tick bites are reported in Sydney, Australia.
2010: There is a range of evidence that ticks are responsible for the IgE response in the United States.
2011: There is extensive evidence that the IgE response is not related to asthma, despite cross-reactions with dog and cat.
2014: Open challenge tests confirm the delay in reactions to red meat.

exacerbation; and a cohort of children in Kenya who showed sensitization to cat despite limited cat exposure. These studies involved extensive investigation of lung function, exhaled nitric oxide levels, histories of asthma symptoms, and serum assay results for IgE antibodies to alpha-gal. Taken together, those studies showed no association between sensitization to alpha-gal and asthma.<sup>28</sup> One caveat is that persons develop this syndrome differently than the typical aeroallergen sensitivity because alpha-gal does not appear to be airborne, and it might be that if given enough time after the onset of symptoms, these persons would have asthma. This would either require a prospective study following patients and seeing how their lung function changes over time when they have a high alpha-gal IgE titer or a retrospective study many years from now after patients have had the disease for years comparing the new lung function with lung function before development of disease.

Previously, we studied an asthmatic cohort from Sweden and found a strong correlation between atopic sensitization to cat allergens and asthma.<sup>29</sup> However, when we examined a population from rural Kenya, we saw high-titer IgE to cat allergens but no association with asthma or atopic disease. What we did not understand was why the level of sensitization to cat was so high in Kenya while exposure to cats was low. A clue to that riddle was provided when we found that patients in the United States with delayed anaphylaxis to red meat had positive skin prick test responses and serum assay results to cat. Further investigation revealed that the alpha-gal-positive patients also had positive results to cat epithelium extracts but not to Fel d 1.<sup>28</sup> On re-examination of the Swedish and Kenyan cohorts, it was discovered that for Sweden, there was a strong correlation between IgE levels to Fel d 1 and IgE levels to cat dander, whereas in the Kenyan population there was no correlation.<sup>28</sup> Instead, there was a strong correlation between IgE levels to cat dander and IgE levels to alpha-gal, thus explaining the apparent high levels of serum IgE to cat we had observed in the Kenyan cohort. Care must be taken when interpreting skin test or serum results in patients who present with symptoms of urticaria, angioedema, and idiopathic anaphylaxis.

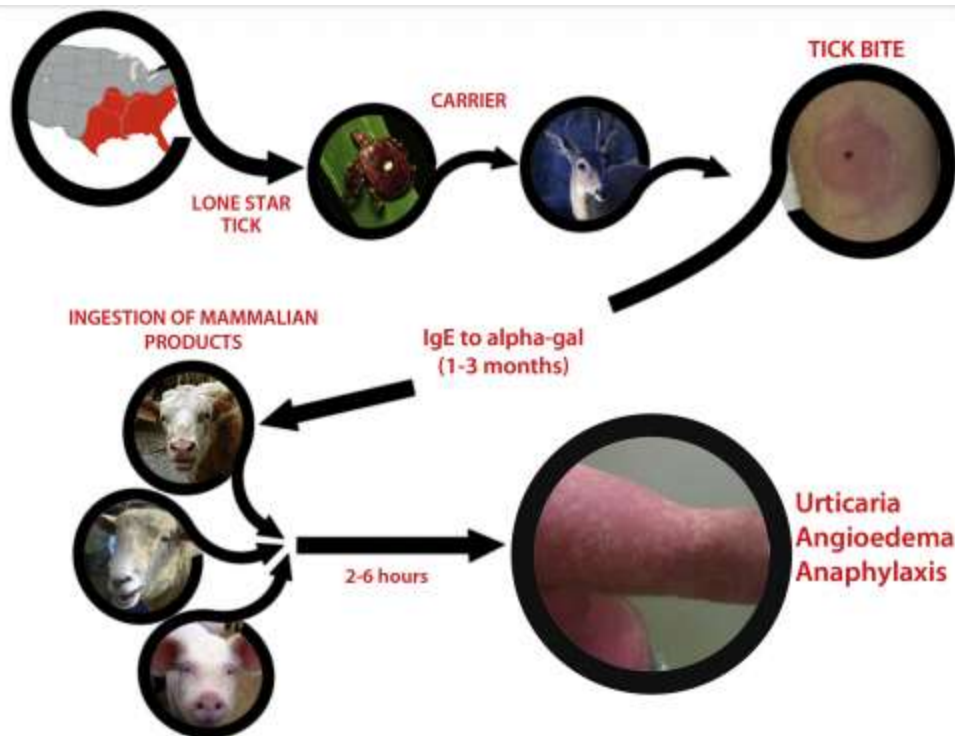
**TABLE II.** Ticks that commonly bite human subjects in countries where IgE to alpha-gal has been reported

	Tick	Outcome
United States	<i>Ixodes scapularis</i> (deer tick)	Lyme disease
	<i>Dermacentor variabilis</i> (dog tick)	RMSF
	<i>Amblyomma americanum</i> (lone star tick)	IgE to alpha-gal, ehrlichiosis, and RMSF
Australia	<i>Ixodes holocyclus</i>	IgE to alpha-gal and/or tick bite-induced anaphylaxis
Europe	<i>Ixodes ricinus</i>	IgE to alpha-gal and Lyme disease
	<i>Argas reflexus</i> (pigeon tick)	Anaphylactic reactions to tick bite

In Zimbabwe Dr Elopi Sibanda, working with colleagues in Austria and Sweden, identified a group of patients who had IgE to cat allergens, which was explained by IgE to alpha-gal.<sup>30</sup> In that report they focused on the potential for IgE to alpha-gal to produce “false-positive” or confusing results for cat allergy. However, equally interesting was the observation that these patients did not report allergic reactions to red meat. In fact, given the evidence that many children and adults in Africa have IgE to alpha-gal, there are remarkably few reports of delayed or other allergic reactions to meat on that continent. Whether this reflects (1) a difference in IgE response, (2) some aspect of the fat content of meat or digestion of meat, or (3) a difference in the response of mast cells is not clear.

#### MECHANISMS OF ANAPHYLAXIS

Currently, it is our belief that the initial step in sensitization to the oligosaccharide alpha-gal is through tick bites. The patients have IgE antibodies to this hapten that is present on all nonprimate mammalian food products. This is comparable with the sensitization that occurs to inhaled plant oligosaccharides, such as MUXF3, a hapten on the glycoproteins of many plant species.<sup>31–33</sup> Unlike alpha-gal, IgE antibodies to these plant-derived cross-reactive carbohydrate determinants have not been shown to contribute to symptoms related to pollen exposure.<sup>31,34</sup> Patients with IgE to alpha-gal typically report symptoms beginning 3 to 5 hours after eating meat. Despite detailed and aggressive questioning, the patients do not recognize any oral or gastrointestinal symptoms less than 2 hours after eating a meal. Similarly, in challenge studies with pork, hives and other symptoms are delayed at least 2 hours after meat ingestion.<sup>35</sup> This is different than the reactions to cetuximab that develop rapidly, in which symptoms often peak within 20 minutes of initial administration of the drug.<sup>1,3</sup> This rapid time frame is similar to the *in vitro* responses of basophils after activation with glycoproteins, such as beef thyroglobulin or cetuximab, which can be detected within 25 minutes. Skin test responses to cetuximab, beef extract, pork sausage, or beef thyroglobulin are also rapid. Thus the delay in response after eating meat does not reflect a delayed response or inability of basophils or mast cells to be activated by these glycoproteins. The obvious explanation is that the oligosaccharide is absorbed from the gut in a form that enters the circulation slowly. Given that alpha-gal is present on both glycoproteins and glycolipids (including chylomicrons), it is our belief that the most



**FIG 3.** Summary of alpha-gal sensitization leading to clinical symptoms of red meat allergy. The southeastern section of the United States is where most of the reactions to red meat have been reported. This region overlaps with the distribution of the lone star tick. The current hypothesis is that persons are bitten by lone star ticks carried by deer into rural and urban areas. After a period of time, IgE to alpha-gal develops. Once IgE to alpha-gal reaches sufficient levels, ingestion of red meat can trigger reactions. Several of the images used in this figure are licensed under a Creative Commons CC BY-NC 2.0 (Attribution-NonCommercial 2.0 Generic) license (Cow: <https://i.flic.kr/p/dgghp> by user Plashing Vole; Deer: <https://i.flic.kr/p/jzWq7> by user Cherry Bream; Sheep: <https://i.flic.kr/p/4WtD> by user Lauren; Tick: <https://i.flic.kr/p/dnNaY> by user Katja Schulz; Pig: <https://i.flic.kr/p/N7gpc> by user Anne).

likely explanation for the delay in symptoms is due to a delay in the appearance of the antigen in the circulation. Because chylomicrons enter the circulation through the thoracic duct after a several-hour process of absorption, repackaging, and transit, mediator release triggered by the accumulated metabolic products (eg, very low-density lipoprotein [VLDL] or low-density lipoprotein [LDL]) might account for the now documented delay. Our studies have shown that during a challenge, circulating basophils assessed *ex vivo* upregulate the expression of CD63 in a time frame similar to that of patients having symptoms.<sup>28</sup> Surprisingly, a proportion of nonallergic control subjects also demonstrated upregulation of CD63, although they do not experience any symptoms. Evidence that basophils and mast cells have receptors for LDL was reported many years ago.<sup>36,37</sup> We postulate that the likely explanation for this enigmatic finding is that although VLDL or LDL can cause basophils to upregulate CD63, the quantity of histamine

released in nonallergic control subjects is not sufficient to cause symptoms. The implication is that LDL particles with alpha-gal on the surface can cause mast cell mediator release but only in subjects with IgE antibodies to alpha-gal. In keeping with this model, 3 of the challenge patients but none of the control subjects had tryptase in their circulation after challenge.<sup>28</sup>

In the United States, delayed allergic reactions are almost uniformly related to eating beef, pork, or lamb, with a minority of cases reporting reactions to milk or cheese. However, in most cases the reactions have followed consumption of more than 100 g of mammalian meat. By contrast, in Europe it is normal to eat meat and organs from a much wider range of mammals. This includes not only horse, goat, and rabbit but also liver, heart, tripe (intestine), or kidney. Two separate groups have reported that reactions to pork kidney can be both severe and more rapid (ie, 2 hours rather than 4 hours).<sup>29,30</sup> In addition, there are increasing anecdotal reports from both the United States and



Europe that drinking alcohol at the same time as eating red meat or kidney can increase the probability of a reaction.

### NATURE OF ALPHA-GAL IgE DEVELOPMENT

Although our data and those of others support the theory that bites from ectoparasitic ticks initiate the development of an IgE response to alpha-gal in human subjects, the mechanistic aspects of this response have not yet been elucidated. There is already extensive evidence that (1) IgE antibody responses can occur outside mature germinal centers,<sup>40,41</sup> (2) that the switch to IgE can occur in B cells locally in the nose,<sup>42</sup> and (3) that antibody responses to oligosaccharides can be or normally are relatively T-cell independent.<sup>43-45</sup> Thus there is a real possibility that the IgE response to alpha-gal involves switching that occurs outside germinal centers, and it is possible that the skin is the site of such a switch. It will be important to establish the extent of rearrangement in the complementarity-determining region, which could provide additional clues regarding the antigen or antigens involved.<sup>45</sup> Previously, it has been documented that persons bitten by the pigeon tick (*Argas reflexus*) can have specific IgE to extracts made from the whole tick body.<sup>46</sup> From preliminary experiments, we have noted that in subjects with IgE to alpha-gal, there appears to be proliferation of a subset of plasmablasts in response to tick extract that was not present in control subjects. In keeping with the observed decreases in IgE levels and reactivity to alpha-gal over time in patients who avoid further tick bites,<sup>17</sup> the formation of plasmablasts could occur in this setting without the development of long-lived plasma cells. Overall, the alpha-gal IgE response has some features resembling an IgM response to an oligosaccharide. Certainly, understanding why exposure to one antigen leads to a long-lived IgE response when another exposure does not would be an important and potentially therapeutically manipulable insight.

### CONCLUSION

The finding that IgE to alpha-gal explains 2 novel forms of anaphylaxis has not only changed several established rules about allergic disease but has also opened up at least 2 new areas of research. The results provide evidence that (1) IgE responses to an oligosaccharide can induce significant or severe allergic symptoms, (2) demonstration of sensitization to this epitope by skin tests often requires both intradermal and skin prick tests, (3) ticks can induce high-titer food-specific IgE responses in adult life, and (4) eating mammalian products carrying this epitope does not give rise to any symptoms during the first hour or more. Like so many new findings, this area of research provides both challenges and opportunities. The delay in onset of symptoms after eating red meat is best explained by delayed arrival of the relevant form of antigen in the circulation, but the question remains as to what form of glycoprotein or, more likely, glycolipid takes 3 hours or more to appear in the circulation.

Finally, the often-rapid production of IgE antibodies to alpha-gal after tick bites provides a striking model of a parasite-induced IgE response (Fig 3). This parasite only enters through the skin, and the tick saliva contains a wide variety of agents that could act as antigens, adjuvants, or both. However, it remains a striking challenge to identify why the response is so strong and why it is directed so consistently against the alpha-gal carbohydrate residue.

### What do we know?

- IgE antibodies specific for the mammalian oligosaccharide alpha-gal are common in a large area of the southeastern United States.
- These IgE antibodies are causally associated with 2 novel forms of allergic reactions: (1) anaphylaxis or urticaria during the first infusion of cetuximab and (2) urticaria, angioedema, or anaphylaxis starting 3 to 5 hours after eating red meat.
- These IgE antibodies in the United States are caused predominantly, if not exclusively, by bites of larval or adult lone star ticks.

### What is still unknown?

- Although deer are the major vector for the relevant ticks in both the United States and Sweden, the increase in deer populations might not be the only or the major cause for an increase in the disease.
- Can the IgE response to tick bites be explained simply by the normal contents of tick saliva, or is it possible that some other symbiotic organism, such as a new *Rickettsia* species, is involved?
- Although the best explanation for the delayed food-induced response to red meat is that it relates to the absorption of lipid particles, it is not clear what form of particle carrying glycolipids or glycoproteins is present in the circulation after 3 to 5 hours.

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## LESSON 5: Student Experimental Design Data Collection & Analysis

### Experimental Design Template

**Part A: To be completed and approved before beginning the investigation**

What question will you explore? \_\_\_\_\_

\_\_\_\_\_

On the basis of your previous laboratory exercise, background knowledge, and research, what is the hypothesis that you will test? \_\_\_\_\_

\_\_\_\_\_

What will be the independent and dependent variables? \_\_\_\_\_

\_\_\_\_\_

What will be the control group(s)? \_\_\_\_\_

\_\_\_\_\_

What equipment and materials will you need (list items and quantity)? \_\_\_\_\_

\_\_\_\_\_

What procedure (step-by-step) will you follow? \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

What safety steps will you follow (equipment and procedures)? \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

How will you collect data? \_\_\_\_\_

\_\_\_\_\_

How will you analyze data? \_\_\_\_\_

\_\_\_\_\_

Teacher approval to begin your investigation: \_\_\_\_\_





## Inquiries in Science®: Behaving Like Animals Kit

Item # 251021 **Exclusive**



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*Grades 9-12.* Give your class an opportunity to investigate animal behavior firsthand. Students become behavioral analysts as they study isopods (pill or sow bugs) and their behavior in a choice chamber. After making observations over time, students adjust environmental conditions to determine the isopods' preferred habitats. Helps you teach the cross-cutting concepts of Cause and Effect and Stability and Change. Kit contains materials for 30 students working in groups of 3.



Pre Lab Expectations-10 points						
	10	8	6	4	2	0
	All assigned prelab background information/questions thoroughly answered and explained. Variables are identified accurately and data tables are created.	All but one of the assigned prelab background/questions is thoroughly answered and explained or variables are not identified accurately.	Three of the assigned prelab background/questions is thoroughly answered and explained.	Two of the assigned prelab background/ questions is thoroughly answered and explained.	One of the assigned prelab background/questions is thoroughly answered and explained.	Not present prior to lab beginning
During Lab Expectations-5 points						
	5	4	3	2	1	0
	Arrive on time, prepared (pre lab complete, closed toed shoes), safely remain on task throughout lab.		One of the requirements for a 5 is missing		Two of the requirements for a 5 is missing	Unexcused absence on day of lab
Graph Expectations-10 points						
	10	8	6	4	2	0
<b>Graph Requirements</b>	All requirements are included (title, labeled y-axis/x-axis, neat data representation) and are accurate.	One of the requirements for a 10 is missing or inaccurate.	Two of the requirements for a 10 are missing or are inaccurate.	Three of the requirements for a 10 are missing or are inaccurate.	Four of the requirements for a 10 are missing or are inaccurate.	Absent from report

Post Lab Expectations-30 points						
	10	8	6	4	2	0
<b>Calculations (if necessary)</b>				A sample step by step calculation has been performed (review specific expectations on lab day) as a sample to show competency.		Absent from report
<b>Conclusion Introduction</b>	The question being asked in the lab investigation is clearly identified, variables (independent, dependent, constant and control group) are correctly identified and discussed.	One of the requirements for a 10 is missing or inaccurate.	One of the requirements for a 10 is missing or inaccurate.	Three of the requirements for a 10 are missing or are inaccurate.	Four of the requirements for a 10 are missing or are inaccurate.	Absent from report
<b>Hypothesis, Data and Discussion</b>	A hypothesis/null hypothesis are provided and identified as either supported or refuted by the data. Relevant data is presented (including units). An explanation of the data (the why) is clearly explained and can be supported with biological principles/knowledge.	Some data is used effectively but the focus may be on just one of the variables or there are some minor errors in the communication of the data. The explanation of the data is present but not detailed enough to receive a 10.	Little data is used effectively or the data is inaccurate. The explanation of the data is minimal or may be biologically inaccurate based on students' expected body of knowledge.	No effort to explain what the data means has been provided.	No data or effort to explain it has been provided.	Absent from report
<b>Real World Connection/ Big Idea/ Previous Content</b>				A logical and descriptive effort has been made to link the lab's purpose/problem, procedure, or conclusion/discussion to one of the big ideas, a real world application or problem or content previously discussed in class. Examples are provided to support the connection or where appropriate reference to outside articles, video clips, scientific journals is provided.	One of the requirements to earn a 4 are missing.	Absent from report

## Lesson 6: Optional Extensions

### Lesson 6: Part 1: Simulated Tick DNA Extraction and PCR



#### Plant Biotechnology: DNA Extraction Kit

Item # 154704

*Intermediate* - Easy to perform; requires some basic training in microbiology.

Extract DNA from wheat germ! No waiting or taking turns! Designed so a class of 30 students can perform the experiment simultaneously. Wheat germ is ground, the cells are lysed, and cellular contents are released. Extracellular protein is digested by enzyme treatment and heating. The DNA is then spooled on a stirring rod. A hot plate and 70 to 95% alcohol are needed but not included.

PRICE: \$101.95

 In stock and available to ship.

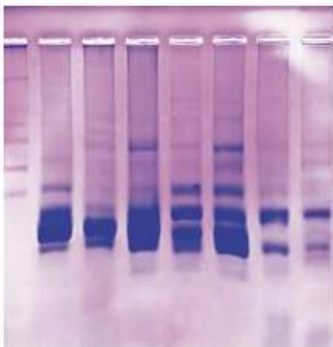
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### Lesson 6: Part 2: Simulated Electrophoresis Fingerprinting and/or BLAST DNA Comparison

Home » Search for: fish dna fingerprinting



#### Fish Protein Fingerprinting on Polyacrylamide Gels Kit (with Perishables)

Item # 211260P



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Explore the evolution of fish species through protein analysis. Students perform gel electrophoresis on extracted muscle protein mixtures from 7 different types of fish. Students compare the protein fingerprints from the 7 different types of fish, hypothesize on the degree of relatedness of the fish, and compare their ideas to a standard evolutionary tree of fish. Comes with perishable material.

**Case Closed**