Potential Lateral Flow Test Design for the Detection of Paralytic Shellfish Poisoning

Taylor Seitz and Chelsea Parrocha

Abstract

A major concern in Alaska is the presence of Paralytic Shellfish Poisoning (PSP). This impacts the communities all along the Alaskan seaboard (which equates to over 1/3 of the US coastline). This has a direct impact on the Alaskan fishing industry, which as of 2009 harvested 52 percent of the commercial seafood in the United States. Due to its influence on the Alaskan economy and sustenance, the study and development of a lateral flow test to detect PSP's notorious toxin (saxitoxin) was implemented. The creation and concept of the lateral flow test is to allow commercial fisheries and local fisherman the ability to access immediate results displaying if their yield is contaminated with PSP. The principals of ELISA were used in the creation of the test. Using chicken antigen as the model compound of detection, the engineering of the dipstick was tested in three phases. Through each phase, alterations in the dipstick involved the manipulation and placement of the blotting and nitrocellulose paper, as well as the placement and amount of antibodies used. All three phases of the test showed design flaws. However, results of the tests demonstrate the progression and improvement of the lateral flow test's design and thus moves the study to a promising future in creating a dipstick that will effectively identify PSP. In the future, with additional time and financing, the further development and perfected design of the lateral flow test is obtainable.

Introduction/Background

One of the unsolved problems in Alaska is an increased prevalence of Paralytic Shellfish Poisoning (PSP). This impacts the communities all along the Alaskan coastline (which is over 1/3 of the US coastline). In addition to South Eastern Alaska, PSP is a common issue around the coastlines of the Northern Atlantic and Pacific Coast of North America.

PSP—also known as "red tide" due to algae causing the water to turn red—is a toxic condition that causes serious paralysis when consuming contaminated aquatic organisms. Organisms that can be affected include mollusks, gastropods, sea birds, zooplankton, whales, herring, salmon, and other fish species.

PSP cannot be cleansed from contaminated seafood, nor can it be removed by heat. If PSP is in the human system symptoms include the following: numbness, tingling, and burning of the perioral region, nausea, fever, rash, and staggering. Some of the most severe cases of PSP lead to immediate respiratory arrest and death (within 24 hours of consumption of contaminated organisms).

Alexandrium Catenella is a dinoflagellate (a type of algae) that produces the saxitoxin that is the cause of PSP. There have been tests (such as ELISA) made to identify the toxin, however the process is lengthy as well as costly.

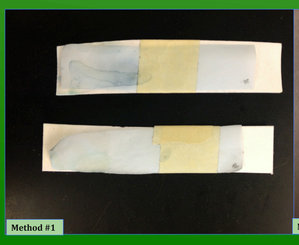
The negative of our experiment will be water, and the positive will be the compound of interest. We hope to see an antibody interactions occur in the dipstick—indicating that our stick works. In the process of developing a dipstick to detect Paralytic Shellfish Poisoning (PSP), we endured three phases of testing. Our model compound of interest was chicken. In the first two tests, we used a conjugated HRP substrate along with a goat anti-chicken antibody. Using chicken as our compound of interest, we used Cy 3-conjugate rabbit anti-chicken antibodies and normal chicken serum. All three test phases of our project had flaws, though our third test was able to display the presence of antibody reactions.

Materials

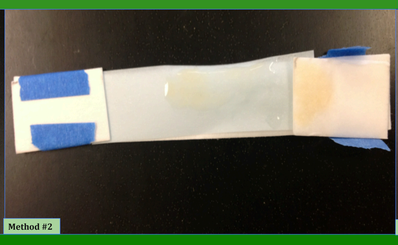
- Nitrocellulose Membrane
- 100% Cotton Fiber Blotting Filter Paper
- Whatman Chromatography Paper
- Primary Goat Anti-Chicken Antibody
- HRP Substrate
- %5 Powdered Milk Solution
- Normal Chicken Serum
- Cy3 conjugate Rabbit Anti-Chicken
- 1X PBS solution
- Micropipettes
- Micro-centrifuge tubes
- Epoxy Glue
- Toothpicks
- Lab tape
- Cardstock
- Laminate
- Glass slides

Methods and Results

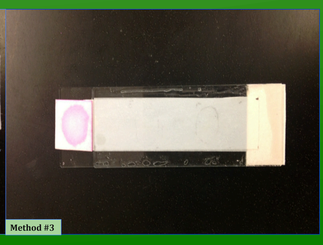
Method #	Methods/Description	Trial #	Date	Results
1	Overall Dimensions: 3cm X 10cm 1. Placed 0.5µl of Primary Antibody on membrane and dried for 30 minutes. 2. Saturated nitrocellulose membrane for 30 minutes in 5% milk solution and dried for 30 minutes. 3. Taped blotting paper under nitrocellulose membrane . 4. Soaked in 250µl 1X antigen solution and dried for 15 minutes. 5. Rinsed with 1X PBS solution. 6. Soaked in 250µl 1X secondary conjugated antibody (HRP) and dried for 15 minutes. 7. Rinsed for PBS solution and soaked in TMB substrate. 8. Let sit 2 minutes for reaction to occur.	1	12/12/12	Nitrocellulose membrane was soaked in milk for to long and blocked the primary antibody from substrate and antigen. The blotting paper too absorbent and did not produce proper wicking.
		2	01/17/13	The test line was present. This method works, but involved surplus steps.
2	Overall Dimensions: 1.5cm X 10cm 1. Laminated cardstock to contain dipstick 2. Blotting paper was secured on top of cardstock 3. 1.0 µl of primary antibody was pipetted on nitrocellulose membrane, and then dried for 15 minutes. 4. Saturated a square of blotting paper with 3.5µl of 1.5g/ml Cy3 conjugated antibodies. 5. Secured saturated conjugate pad on one end of the dipstick. 6. Secured blotting absorbent pad on the other end. 7. Pipetted 0.5ml of 2X chicken serum as antigen sample and waited for liquid to flow down the nitrocellulose membrane..	1	02/12/13	N/A, no reaction due to the antigen sample not traveling properly down the nitrocellulose membrane.
		2	02/19/13	N/A, the lab tape securing different elements of the design blocked the sample from traveling.
		3	02/21/13	N/A, there was too small an amount of unconjugated primary antibodies.
3	Overall Dimensions: 9cm X 2.5cm 1. Placed 15µl of 50X primary rabbit anti chicken antibodies in the center of nitrocellulose membrane. 2. Secured nitrocellulose membrane and an identical piece of chromatography paper onto glass slide. 3. Secured second glass slide staggered onto of the other. 4. Secured absorbent pads to both ends of the dipstick. 5. Waited 20 minutes for epoxy to dry completely. 6. Mixed 20µl of primary conjugated antibodies with 15µl of 100% chicken serum in a microcentrifuge tube and inverted the tube until mixed completely. 7. Applied antibody and chicken serum mixture to one absorbent pad. 8. Slowly added 2ml of dH_2O to the absorbent pad to wash the antibody and chicken serum mixture down the nitrocellulose membrane between the glass slides. 9. Once the left over water reached the opposite absorbent pad, the results were recorded.	1	02/27/13	N/A, there was too small an amount of unconjugated primary antibodies.
		2	03/04/13	N/A, the blotting paper and chromatography paper was to absorbent, causing the loose conjugated antibodies not to travel down the dipstick.



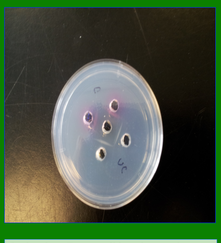
Method #1



Method #2



Method #3



Ouchterlony Test to validate the reactions between antibodies and antigen.

Discussion

As conclusion to the experiments, progress was displayed throughout the different designs. The first method displayed positive and promising results, however the model didn't have the desired procedures. The second method was a large step from the first and came closer to the intended outcome. Since the second method was disorganized a third method was implemented to improve all the previous flaws. The third and final design of the lateral flow test was much more successful, as it addressed all previous issues in the first and second model. It was not sufficent enough to fulfill capillary action of the test.

Although the majority of our designs were problematic, we were able to demonstrate a progression of improvement in the method and development of the dipstick. Through out experiment, we were able to learn that soaking the nitrocellulose paper in milk does not improve the visual presence of antibody interactions.

Due to the inability of visibly seeing antibody interaction during methods 1 and 2, we believed that our antibodies have expired. However, after performing an Ouchterlony test, it proved that our antibodies were not expired and therefore it was our model that needed adjusting. We came to the conclusion that the only problem in the third design (method 3) was due to the blotting paper; cotton fiber blotting paper was used in this model, and proved to be too absorbent.

Originally we intended to do our project using the actual saxitoxin, however due to our limited resources, we were not able to pursue our original intent. In the future, we intend to continue this project in hopes for perfecting the design for a dipstick to detect PSP.

The next challenge and phase of this experiment is to optimize a final test to detect saxitoxin in PSP. Ideally instead of Cy3 conjugates, a colloidal gold or silver will be used to display a more violent reaction. Also instead of 100% cotton wicking paper, a glass fiber blotting and filter paper will be utilized to produce a stronger capillary action. With additional resources, our experience, and utilization of the methods used to create the lateral flow test, the creation of a new lateral flow test to detect the presence of PSP is in the near future.

References

Center for Disease Control and Prevention (CDC). (2011, November 18). *Morbidity and Mortality Weekly Report*. Retrieved 2012, from Center for Disease Control and Prevention (CDC): http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6045a3.htm

Fleming, L. E. (2012, July 31). *Paralytic Shellfish Poisoning*. Retrieved from Harmful Algae: http://www.whoi.edu/redtide/page.do?pid=9679&tid=523&cid=27690

Jester, R. J., Baugh, K. A., & Lefebvre, K. A. (2009, January 7). *Presence of Alexandrium catenella and paralytic shellfish toxins in finfish, shellfish and rock crabs in Monterey Bay, California, USA*. Retrieved from eScholarship: http://escholarship.org/uc/item/1cf1p03h#page-1

National Oceanic and Atmospheric Administration (NOAA). (2012). *Paralytic Shellfish Poisoning*. Retrieved 2012, from Northwest Fisheries Science Center: http://www.nwfsc.noaa.gov/hab/habs_toxins/marine_biotoxins/psp/index.html

Russell E. Colemana, J. F. (2000). Development and Evaluation of a Dipstick Assay for Detection ofPlasmodium falciparumandP. vivaxsporozoites in Mosquitoes (Diptera: Culicidae). *Journal of Medical Entomology* , 37 (4), 581-587.