**Scenario**

You and your team have just arrived on an island in the Philippines as part of a field research experience. The island laboratory is staffed by a famous researcher who is in charge of a summer learning program. The head researcher, Dr. C. Shore, is a world famous specialist in marine biology and a well-respected expert on animal toxins. When you arrive at the lab, you find Dr. C. Shore slumped on the floor. After turning over the victim, you see that she has a syringe sticking out of her rump. It is apparent that Dr. C. Shore sat on the syringe, injecting most of the contents, an unknown fish venom, into her body. Located in the work area of the researcher are many vials of fish venoms and antivenins labeled with the corresponding species, but it is unclear which vial Dr. C. Shore was working with.

**Team Task**

Your job is to figure out which fish toxin Dr. C. Shore was working with and administer the correct antidote. Your time is limited because the treatment must be administered as quickly as possible. Also limited is the amount of liquid left in the syringe and the amount of testing materials in the lab, so your team can only test two antivenoms.

Your first order of business is to narrow the list of five possible fish venoms down to the two most likely. Use the two antivenoms you chose in order to run a single test using the small sample you have remaining in the syringe. Hopefully one of these bottles of antidote is the correct one but there are no guarantees.

**Victim’s Symptoms**

Dr. C. Shore is breathing weakly. Her heart rate is elevated and her skin feels feverish. Her eyes are closed but not tightly. When you move around her you can see the movement of her eyes as if they might be tracking you. Dr. C. Shore is also somewhat rigid, not limp as when someone is sleeping but rather her muscles display some contraction. Clenched lightly in Dr. C. Shore’s hand is a UPS mailing label for a company in the Dominican Republic.
Habitat

Common to the subtropical parts of the Atlantic, Indian and Pacific Oceans. This species blends in well with certain corals located near the sea floor.

Toxin

A neurotoxin called Tetrodotoxin (TTX).

Toxin Effects

- Dizziness and vomiting
- Numbness and prickling all over the body
- Rapid heart rate and decreased blood pressure
- Difficulty in walking occurs and, in some cases, paralysis
- The toxin paralyzes diaphragm muscles and victim stops breathing
- Patients are commonly awake for up to two hours prior to death with no ability to communicate

Treatment

- Activated Charcoal, first-aid, and life-support are recommended
- Antivenom available, but has not been readily tested on humans
**FISH PROFILE #2**

**Red Scorpionfish • Scorpaena scrofa**

**Habitat**
This species lives on or near the bottom of the ocean waters in crevices, caves, and under overhangs. They range from the Red Sea, Pacific Ocean from Australia to Hawaii.

**Toxin**
Red Scorpionfish have venomous spines that contain a neurotoxin which directly affects the nervous system.

**Toxin Effects**
- Severe pain and swelling at the site of the sting; swelling can spread to affect an entire arm or leg within minutes.
- Blood toxicity
- Heart rate changes—either fast or slow
- Paralysis
- Muscle spasms and/or seizures
- Shortness of breath

**Treatment**
- Antivenom available
Habitat
This species lives primarily above the Tropic of Capricorn. It is found in shallow tropical waters of the Pacific and Indian coasts from the Red Sea to the Great Barrier Reef.

Toxin
One of the most venomous fishes in the world. Dorsal spines release the venom from sacs along the spine. The venom is a neurotoxin, a mixture of proteins including hemolytic stonustoxin and the cardioactive cardioleputin.

Toxin Effects
- Severe pain at sting site; the pain is said to be so severe that affected persons scream to have the affected limb amputated
- Whitened color of the area around the site of the sting
- Difficulty breathing
- Paralysis
- Muscle twitching and seizures
- Nerve and tissue damage often results

Treatment
- Application of heat to the affected area using hot water at a temperature no lower than 45 °C (113 °F); heat applied to the injured area has been found to destroy Stone Fish venom
- Antivenom available
### Habitat
This species lives in the reefs of the Indian and Western Pacific Oceans. They are found as far north as southern Japan and as far south as Australia.

### Toxin
A neurotoxin composed of heat-sensitive proteins. The toxin can remain active in the spines for days so even discarded spines remain dangerous.

### Toxin Effects
- Severe pain at the sting site and headaches
- Nausea and vomiting
- Fever
- Decreased heart rate
- Rapid swelling of the joints can occur making movement difficult, if not impossible

### Treatment
- The best treatment is to immerse the wound or run it under very hot water for at least 30 to 40 minutes. The venom’s protein will be broken down by heat.
- Antivenom available
**Habitat**

This species is found in the western Atlantic Ocean from North Carolina and the northern Gulf of Mexico, south to the northern coast of South America. It is absent in the West Indies.

**Toxin**

A neurotoxin called Tetrodotoxin (TTX). Stargazers have the ability to produce electrical currents.

**Toxin Effects**

- Intense, throbbing pain
- Puncture is accompanied by swelling, redness, and heat
- Symptoms of shock
- Difficulty in walking occurs and, in some cases, paralysis
- The toxin paralyzes diaphragm muscles and victim stops breathing
- Patients are commonly awake for up to two hours prior to death with no ability to communicate

**Treatment**

- Activated Charcoal, first-aid, and life-support are recommended
- Antivenom available, but has not been readily tested on humans
Lab Procedure

ELISA Simulation Test

1. Using one clean pipette, carefully administer 3 drops of VENOM into the wells 1, 2, & 3 for each of the letters A, B, C, D, E

2. Using a clean pipette, add 3 drops of positive control to wells A1, A2, & A3

3. Using a clean pipette, add 3 drops of negative control to wells B1, B2, & B3

4. Using a clean pipette, add 3 drops of your first antivenom guess (fish 1). Add these drops to wells C1, C2, and C3

5. Using a clean pipette, add 3 drops of your second antivenom guess (fish 2). Add these drops to wells D1, D2, and D3

6. Using a clean pipette, add 3 drops of the Simulated Secondary Antibody to wells. 1, 2, & 3 of rows A, B, C, D, (E). Basically, add to every well that you are using.

7. Using a clean pipette, add 3 drops of Chromogen to wells. 1, 2, & 3 of rows A, B, C, D, (E). Basically, add to every well that you are using.

8. All of the reaction wells will turn green when the Chromogen is added. A change from green to light purple is a positive result. Refer to your control wells when making your determination.

9. There will be a time delay and a time limit during which you may make your observations of a positive or negative result. For this lab we may assume that the reaction will happen in a minimum of three minutes and not take more than 10 minutes. So your data/observation will have to be between 3 and 10 minutes. Any observation/data noticed before 3 minutes or after 10 minutes must be disregarded as inaccurate.

10. You should get the same results in each of the three wells.
**The Case of the Mystery Fish Toxin - Student Handout**

**Part 1: Introduction**

**Procedure:**
- Read The Case page (scenario, team task, victim’s symptoms).
- Answer questions 1 – 4.
- Read all Fish Profile pages.
- Rank the fish/venoms from most likely to least likely responsible for Dr. C. Shore’s condition (1 = most likely and 5 = least likely) and complete Table 1

1. What does your research team need to figure out?

2. How many fish venoms does your research team need to test?

3. How should your research team narrow down the possible toxins?

4. What are Dr. C. Shore’s symptoms?

Table 1: Rank the Fish/Venoms and explain the reasoning for your ranking.

<table>
<thead>
<tr>
<th>Fish/Venom</th>
<th>Reasoning</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
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<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>
Part 2: ELISA Simulation Test

Procedure:
• Read the Lab Procedure page.
• Answer questions 1-8.

Preparing for the ELISA Simulation Test
1. Which two fish toxins are you testing (most likely to be affecting the victim)?

Fish/venom 1 = ____________________ Fish/venom 2 = ____________________

2. Using the Lab Procedure directions, label the diagram below.
In each well (circle) of the plate, write what you will be administering using the codes below.

\[ V = \text{Venom} \quad + = \text{positive control} \quad - = \text{negative control} \]
\[ 1 = \text{antivenin 1} \quad 2 = \text{antivenin 2} \quad \text{SSA} = \text{Simulated Secondary Antibody} \]

C = Chromogen

3. Predict what color each well will turn and color in the diagram below. Use highlighters so you can still read your writing.
4. What is the purpose of the Chromogen?

5. What color indicates a positive result?

6. What color indicates a negative result?

7. How long will it take for a color change to occur?

8. How long should your research team wait before recording your results?
Part 3: Conducting the ELISA Simulation Test

Procedure:
- Conduct the ELISA simulation test.
- Record your results in the diagram below.
  - Color each well with a colored pencil OR
  - Draw a + or a - in each well
- Answer questions 1–4.

1. According to your ELISA simulation test results, which venom was Dr. C. Shore accidentally injected with?

2. Was your original prediction correct?

3. Would you want to distribute the antivenin without doing an ELISA simulation test first? Explain why or why not.

4. Suggest two other ways the ELISA simulation test could be used.