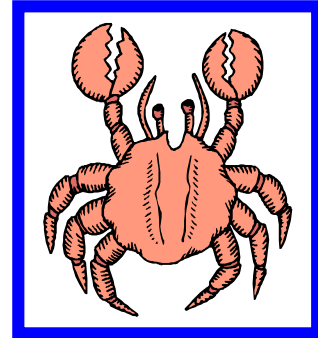


Wealthy Miser, Edgar Earp, dies from poison. Family members are suspects!

Edgar Earp, well known entrepreneur and third cousin to the famous Marshall Wyatt Earp, died last weekend of what the County Coroner says was “an acute allergic reaction to the proteins found in crab meat, specifically, Alaska King Crab.”



Earp has lived for the past eighty years in a decaying old mansion outside Tombstone Arizona. He is reported to be worth several million dollars, but has lived in near seclusion allowing family members to visit the formerly palatial estate only once yearly for his annual birthday party. A miser, he insists that family members bring food for the potluck lunch, rather than catering the event or using his household staff to prepare food. He insists that all family members prepare the food themselves as he has an aversion to store bought or restaurant prepared food and he refuses to spend any money feeding his relatives.

For years, he has hinted to each one of his relatives that they alone will inherit his money when he dies. To their dismay, Uncle Edgar, as he is called by all family members, was in the 90's and seemed to be as healthy as ever. He has never shared any of his suspected wealth with any of his relatives, insisting they should take care of themselves.

The County Coroner reported that family members were summoned to Tombstone for Uncle Edgar's annual potluck birthday party last Saturday. Relatives Dan and Mary each brought a crab dish, as this is the birthday boy's favorite food and they hoped to court his favor. However, they knew to use imitation crabmeat as it was well known that Uncle Edgar has a deadly allergy to real crabmeat.

According to an eye-witness, the food was placed around the table in the great dining hall and as the meal progressed, Uncle Edgar tried several bites of each offering while the rest of the family looked longingly for signs of heart failure as he wobbled around the tables. “Suddenly, the crusty old man stood up, clutched his throat and with an accusing look down the table, fell to the floor, dead!”

You are a newly hired forensic scientist for Wild West County with a well equipped lab. Your job is to identify the murderer by determining which if any of the dishes contain real crabmeat. You will do this by comparing the fragments of protein found in Alaska King Crab and the imitation crabmeat with the crabmeat found in Dan's and Mary's dishes.

As a forensic scientist you must know the following:

How to weigh samples and measure fluids.

Use of microcentrifuge and electrophoresis chambers.

Back in the Laboratory:

You will have the following materials to use:

Mortar and Pestle	plastic spoon
1.5 ml microcentrifuge tubes	Plastic transfer pipettes
Water bath (boiling water)	Dissecting needle
Electrophoresis Chamber	metric ruler
Coomassie Blue staining solution	SDS –agarose gel
Laemmli buffer	10µl micropipette
Sample Preparation Buffer	Distilled water
Destaining solution	Alaska King Crabmeat
Extracts from Dan's and Mary's seafood dish	Imitation Crabmeat

When you are ready at your laboratory station and have collected all materials and have met your supervisor's safety requirements, you can attempt to help solve this mystery. You must determine if either Dan or Mary, both or neither is guilty of feeding real crabmeat to Uncle Edgar, causing his death. The outcome of an arrest depends on your results. You must be prepared to defend your procedure and outcome as attorneys may question every step.

Prepare extracts:

- Step 1. Obtain about a centimeter cube of real crab tissue.
Weigh exactly 1.0g of the tissue and place it in a clean mortar with 2.0 ml of distilled water and grind it to a fine paste.
Add 3.0 ml sample preparation buffer and stir with a plastic spoon
Scrape 1.0 ml of the sample into a 1.5 ml microcentrifuge tube and label R for real. Rinse the mortar and pestle thoroughly, with water AND distilled water.
- Step 2. Repeat step 1 with imitation crab meat and label F for fake.
- Step 3. Place tubes in microcentrifuge in balanced configuration and centrifuge 5 minutes at 3000 rpm to “pellet” the solids.
- Step 4. Use a plastic transfer pipette to remove 0.5 ml of the liquid (supernatant) above the crab tissue pellet and place it into a new microcentrifuge tube labeled (R). Repeat the process with a clean transfer pipette for the imitation crabmeat (F).
- Step 5. Obtain extracts of Dan's and Mary's extracts from your supervisor.
- Step 6. Poke a hole in the lid of each tube with a dissecting needle to allow air to escape during heating. Place the tubes in a floating microcentrifuge tube rack in a boiling water bath for 3 minutes to denature (separate) all the proteins.

Run Samples on Electrophoresis Gels:

Step 7. Obtain an SDS-agarose gel from the refrigerator. Place it in the electrophoresis chamber with the wells positioned at the negative end of the gel box (run to red). Fill the electrophoresis chamber gently with Laemmli buffer until the top of the gel is just covered. (Do not overflow!)

Step 8. Load 10 μ l of each sample
In order indicated.

A = Real crabmeat
B = Imitation crabmeat
C = Mary's extract
D = Dan's extract

<input type="checkbox"/>	A
<input type="checkbox"/>	B
<input type="checkbox"/>	C
<input type="checkbox"/>	D

Step 9. Electrophorese at 75 volts for approximately 45 minutes or 125 volts for 20 minutes. Sample Preparation Buffer serves as the tracking dye, Do not allow the tracking dye to go off the edge of the gel. Turn off the power supply to the chamber and unplug all wires.

Stain the gel:

Step 10. Remove the gel from the electrophoresis chamber, place it in a staining tray and cover with Coomassie Blue staining solution for 2-5 minutes. Pour the Coomassie stain back into the original container after staining for reuse.

Step 11. Cover the gel with destaining solution. Change the destaining solution several times. If necessary, destain overnight. Use paper towels to absorb the stain.

Step 12. Bands should be visible after destaining. Examine the banding pattern over a white light box such as an overhead projector. Be careful to make accurate measurements of the distance from wells. Sketch your results below:

<input type="checkbox"/>	
<input type="checkbox"/>	
<input type="checkbox"/>	
<input type="checkbox"/>	

Your expert analysis:

1. How long and at what voltage did you run the gels? _____

2. What would happen if you ran the gel too long at a high voltage? _____

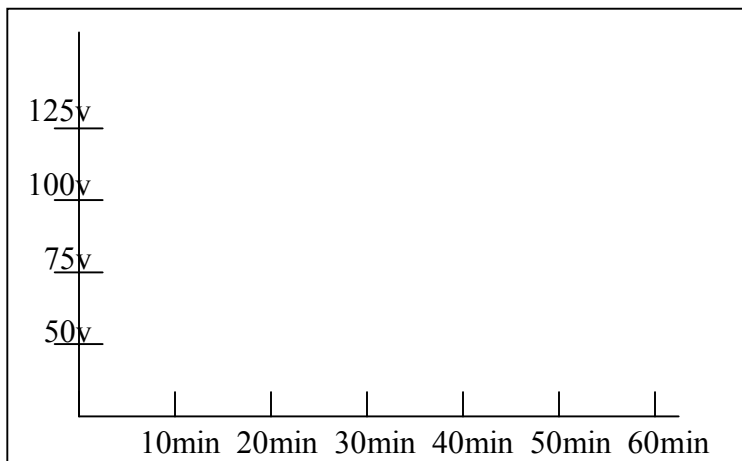
3. Which are the knowns and which are the unknowns in this activity. Write a U or K.
Real Crab _____ Imitation Crab _____ Dan's Extract _____ Mary's Extract _____

4. What did you do to avoid contamination of evidence? _____

5. Why use distilled water, rather than just tap water? _____

6. What were some of the measurements you made and why was it necessary to be careful in measurements? _____

7. If the gel runs at 75 volts for 45 minutes or at 125 volts for 20 minutes, how long would you run the gel at 100 volts? Use the graph below to determine your answer.



8. According to YOUR results:
Dan's extract contained _____ Mary's extract contained _____

9. Who should be arrested for the murder of Uncle Edgar? _____

10. How else might someone else have slipped crabmeat to Uncle Edgar? _____



**Wealthy Miser, poisoned at Birthday Lunch!
Family members are suspects!**

Seafood Forensics

**(Adapted from the Shoestring Biotechnology Project of NABT
and Vickie Vaughan, Western Heights High School, OKC)**