Mycotoxin ELISA Testing **Name:**______________________________ **Group #:**_______

Objective:

Hypothesis:

Experimental Design:

Table 1. Samples for Testing with ELISA Kit

Sample Number	Sample Name
NEOGEN Control	**1ppm DON**
Control	**Trilogy 5ppm Barley**
Control	**Trilogy 0ppm Wheat**
1	White Rice
2	Corn Meal
3	White Flour
4	Ground Corn Field Sample
5	Ground Sorghum Field Sample
6	Fritos
7	Tortilla Chips
8	Bran Cereal
9	Grape Nuts Cereal
10	Wheat Thins
11	Animal Feed- Corn Glucose
12	Dog Food
13	Wheat
14	Barley
15	Organic Flour

1. Each group must test the ***control samples*** seen above in Table 1. Identify the positive and negative control.

   Trilogy 5ppm Barley: _________________________
   Trilogy 0ppm Wheat: _________________________

2. Each group must sign up for 3 other unknown samples on a sheet in the front of the classroom, each sample can only be tested twice (signups on a first come first serve basis). List the unknown samples that your group is testing in Table 2.

Table 2. Unknown Samples Being Tested

Sample Number	Sample Name

3. Weigh out 1g of each ground sample and transfer into **labeled** 15mL vials.
4. Measure 5mL of water in a graduated cylinder and add to the sample vials and shake vigorously for 3 minutes making sure it is well mixed.
5. Place vials in rack and let them settle and separate for 10-15 minutes or until there is enough supernatant. While samples are settling, set up extraction funnels and filter paper.
6. When needed separation is present in the vials, using the 200µL-1mL pipette, extract the supernatant and filter it in the extraction setup.
7. Label red mixing wells and clear wells with following conventions
    i. "C": NEOGEN Control
    ii. "+": positive control
    iii. "-": negative control
    iv. "unknown #": unknown sample number
    v. "unknown #": unknown sample number
    vi. "unknown #": unknown sample number
8. After extractions have filtered, red-mixing wells will be used.
9. Swirl the blue labeled conjugate reagent bottle, set pipette to 100µL and add reagent to each well. Discard the pipette tip.
10. Using a new pipette tip, add 100µL of the NEOGEN control from the yellow labeled bottle to the well labeled "C". Pipette this up and down to make sure conjugate and sample are well mixed. Discard tip.
11. Using a new pipette tip, add 100µL of the first extraction sample into it's corresponding labeled well and pipette this up and down to mix. Repeat this for all 5 extraction samples. *Make sure to change the pipette tip between each sample.*
12. Using a new tip for each, transfer 100µL from each red marked well into the corresponding antibody coated (clear) well.
13. Mix by sliding the wells back and forth on a flat surface for 10-20 seconds without splashing reagents. Wait 5 minutes.
14. Initial reaction is complete. Shake out the contents of the antibody-coated wells.
15. Fill each well with distilled water and shake out 5 times. After shaking out the last time, remove all droplets by turning wells upside down and tapping on paper towel.
16. Swirl the green labeled substrate reagent bottle and add 100µL of reagent to each well. Discard the tip.
17. Mix by sliding the wells back and forth on a flat surface for 10-20 seconds without splashing reagents. Wait 5 minutes.
18. Swirl the red labeled Red Stop reagent and add 100µL of the reagent to each well. Mix by sliding on flat surface and observe color change.

*Adaptations made from *NEOGEN Agri-Screen for DON kit manual*

Mycotoxin ELISA Testing **Name:________________________________** **Group #:_______**

Results:

Sample Name						
Observation						
Interpretation						

1. Compare your controls to another group. Do they differ? If so, what are some reasons? Could this affect how you interpret your results of unknown samples?

2. Were the results what you expected? Did they match your hypothesis? Why is making a hypothesis in the beginning of an experiment design important?

3. Possible sources of error in experiment:

4. Ways to improve this experiment in the future: