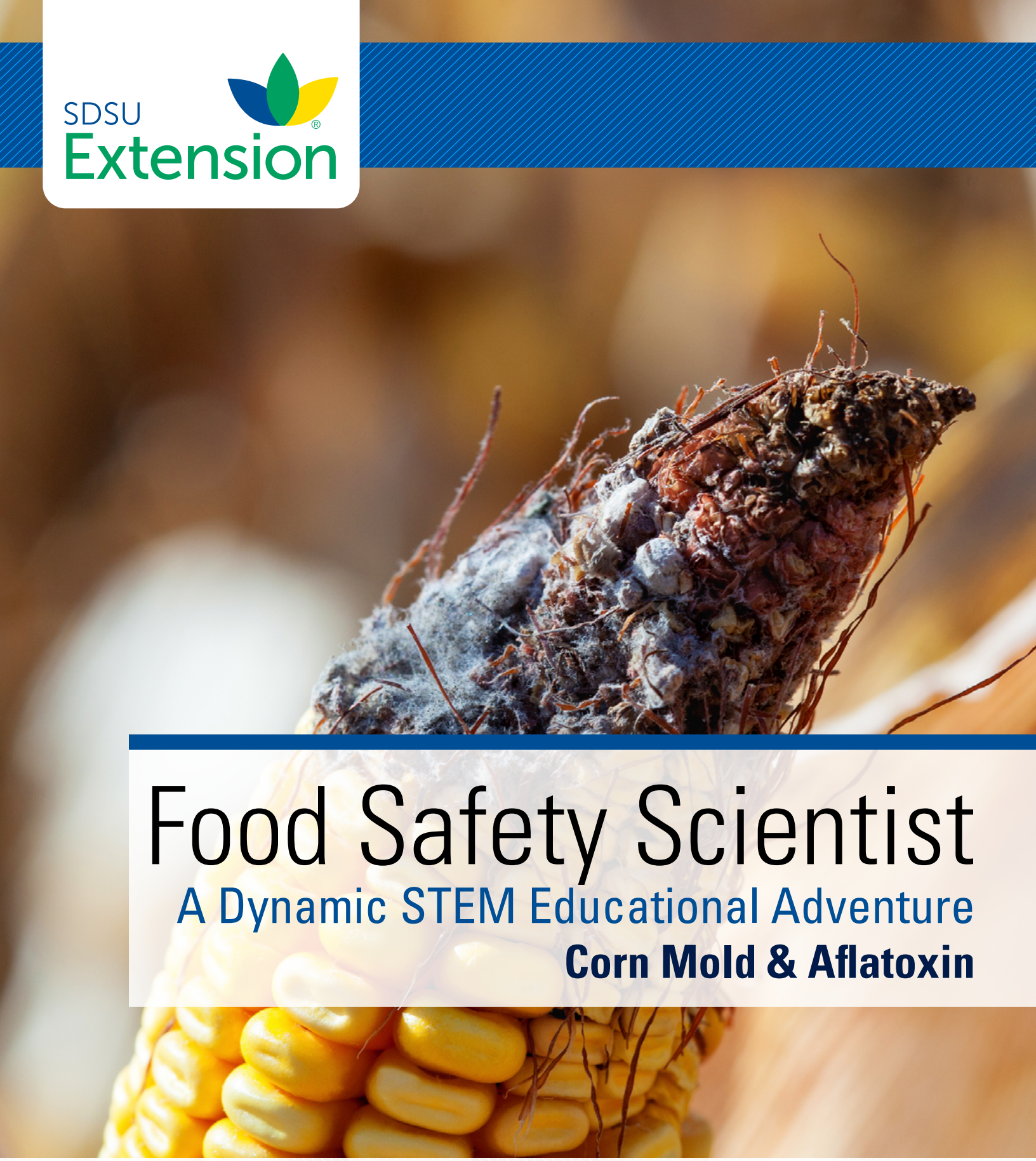
SDSU Extension



Food Safety Scientist

A Dynamic STEM Educational Adventure

Corn Mold & Aflatoxin

Department of **Dairy & Food Science**

College of **Agriculture, Food & Environmental Sciences**

Acknowledgements:

Curriculum:

Food Safety Scientist: A Dynamic STEM Educational Adventure
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Unit: Bacteria – The Good, The Bad and The Ugly

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Unit: Corn Mold and Aflatoxin

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South Dakota State University (SDSU) and New Mexico State University (NMSU) produced this project as part of a Higher Education Challenge Grant "Innovative STEM instruction techniques to increase the number and diversity of students in food safety related majors". SDSU served as the lead institution for the overall project developing the content and curriculum with NMSU leading the digital creation of classroom media, animations and interactive educational technologies. North Dakota State University serves as the outside evaluator of the project. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2011-38411-30625 from the USDA National Institute of Food and Agriculture. This project was supported in part by the USDA NIFA grant numbers 2008-38411-19055, NDSU serving as the lead institution with SDSU and NMSU as sub-awardees. Virtual Lab creative presentation © 2010 The NMSU Board of Regents

Food Safety Scientist

A Dynamic STEM Educational Adventure

Real-life situations provide some of the greatest opportunities for students to learn how science impacts their life. Explore the common science and technological concepts in the development of a safe food supply within the classroom through the Food Safety Scientist Curriculum.

Engaging

The Food Safety Scientist Curriculum is intended to enhance the regular classroom curriculum. Educators are encouraged to pick and choose from the various educational tools to engage students in the STEM related field of food safety.

Scientists Needed

Food safety requires the need for scientists in the research laboratory, production practices, regulatory agencies, veterinarians, food product development and processing, meeting the demands of feeding the world, as well as preparing and serving food within the home. Educators in Science, Agriculture, and Family and Consumer Sciences utilize the components of the curriculum to help students explore STEM careers that keep food healthy and safe.

Free Curriculum

The curriculum is entirely free. Educators can access the website, download materials they want to use in their educational setting and change them to meet their needs. Components of the curriculum have been used in formal and informal educational settings as well as with various age levels. For example, the virtual labs of gram staining and using a microscope have been used with middle school students as well as introductory microbiology courses at colleges and universities across the United States.

Curriculum Components

The curriculum enhancement tool Food Safety Scientist includes the following components:

1. Unit Guide to assist teachers in identifying the goals, objectives and standards (Next Generation Science Standards, and Career and Technical Education Classes) for each unit and individual learning experiences.
2. Interactive Virtual Labs that include real-life situations that bring science, technology, engineering and math into the delivery of a safe food supply.
3. Hands-on laboratory experiences that compliment the virtual labs. Providing the opportunity for students to gain real-life experiences and a greater understanding of the applications of STEM for food safety scientists.
4. Exposure to careers related to the agricultural and food safety sciences.
5. Discussion Guides for teachers to empower students to explore the various scientific concepts that are utilized to develop a safe food system.

Contents of Unit

Corn Mold & Aflatoxin

*The Virtual Lab "Testing for Corn Mold" is a critical component to this unit.

This unit was created to expose students to a career that has a connection to food safety (agronomist, producers, research – ELIZA testing, lab technicians). All the units are developed for the teacher to pick and choose what they want to use. The enrichment activities are developed with a format that allows for educators to adapt for their purposes.

Overall Goal:

Increase the number of students that consider food safety and related fields as a career path

Enrichment Activity	Time Allowed	Objectives Applied*	Standards*
Power point Presentation: Importance & Analysis of Aflatoxin in Corn	20 minutes	1,2,3,5	FCS 8.2.1, FCS 9.2, FCS 9.2.1 FCS 9.5, FCS 9.5.7, ITA7.2, AgP 3.4, FS 1.2, FS 2.2, AS 1.1, HS-ETS1-1
Virtual Lab: Corn Mold Toxins	10 minutes	1,2,3,4,5,6	FCS 8, FCS 9, ITA7.2, AgP 1.2, AgP 1.3, FS 2.2, HS-ETS1-1
Hands on: Testing Ground Corn Samples for Aflatoxin	40 minutes	1,2,3,4,5,6	FCS 8, FCS 9, AgP 1.3, AgP 2.1, AgP 3.4, FS 2.2, AS 1.1, HS-ETS1-1
Class Discussion	20 minutes	1,2,3,4,5,6	FCS 8, ITA7.2, Ag1.2, AgP1.3,AgP 2.1, AS1.1, AgP 3.4, HS-ETS1-1
Power point Presentation: ELISA-Applications & Technology	30 minutes	2,5,6	FCS 9.2,FCS 9.5,AgP 2.1
Homework Assignment: Use of ELISA & Lateral Flow Devices	*Out of Class Assignment or 35-40 minutes in class	1,2	FCS 8, FCS 9.2,FCS 9.5, AgP 2.1

Increase the number of students that consider food safety and related fields as a career path

Note: FCS – Family Consumer Sciences; Ag – Agriculture; AgP – Agriculture Processing Technology; AS – Animal Science: HS-ETS – High School- Engineering Technology Science

*See the following information to identify standards & objectives

Performance Objectives of Students

1. Express an understanding of the monitoring of the food supply for safety and quality.
2. Increase knowledge regarding food microbiology.
3. Students will make inferences and interpretations from knowledge gained regarding the science related to monitoring the safety of the food supply.
4. Students will examine the overall safety of the food supply from the farm to table.
5. Students will describe the various types of safe food handling practices and monitoring of the food supply.
6. Students will identify and give examples of various careers that support the safety of the food supply.
7. Students will evaluate their competency in food safety and related fields.

Standards:

Family and Consumer Sciences

Standard 8: Integrate knowledge, skills, and practices required for careers in food production and services

8.2.1: Determine pathogens found in food and their roles in causing illness

Standard 9: Integrate knowledge, skills, and practices required for careers in food science, dietetics, and nutrition

9.2: Apply risk management procedures for food safety, food testing, and sanitation.

9.2.1: Determine factors that contribute to foodborne illness

9.5: Demonstrate use of current technology in food product development and marketing

9.5.7: Conducting testing for safety of food products, utilizing available technology.

Agriculture

ITA7.1 Illustrate how raw commodities become table-ready food products

AgP 1.2 Discuss how food safety is addressed in the food processing industry

AgP 1.3 Explain how regulatory agencies in the food industry work to protect consumers

AgP 2.1 Translate regulatory procedures as they apply to food processing

AgP 3.4 Process food safely

AN6.1 Compare and contrast consumer concerns related to animal food products.

i.e.: Debate pasteurization of milk products

FS 1.2 Identify industry organizations and their impact on the food industry

FS 2.2 Apply safety and sanitation practices used in the food industry

AS 1.1 Appraise the fundamentals of the agriculture industry and its impact in the world

AS 6.1 Demonstrate how to best maintain animal health

Science (Next Generation Science Standards)

HS-ETS1-1- Analyze a major global challenge to specify qualitative and quantitative criteria and constraints for solutions that account for societal needs and wants

Corn Mold Teacher Resource Sheet

Materials Needed for "Corn Mold Lab" (Per lab group or student)

1 or 2 Aflatoxin test strips
Fixed volume transfer pipette (one for each test strip)
Reaction vial (one for each test strip)
corn sample – ground (10 grams)
4 mL 70% methanol or 50% ethanol solution
plastic (or glass) sample container with tight fitting lid.
Graduated cylinder (10 or 25 ml)
Tap water

Notes

Read the discussion guide while planning this lesson. Many discussion points about the evaluation of food safety are presented.

Read "The Importance and Analysis of Aflatoxin in Corn" document for background information about Aflatoxin and food safety.

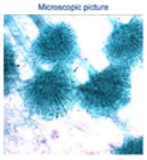
More information about Aflatoxin can be found on the following websites: http://www.extension.iastate.edu/publications/pm1800.pdf.

Use QuickTox™ Kit developed by EnviroLogix Company.

- There are several companies that manufacture ELISA test kits to test for the presence of aflatoxin in corn grains.
- You can obtain additional test kits by contacting the company at Envirologix.com.

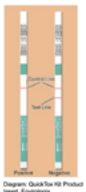
Power point Presentation: Procedure for QuickTox™ Kit for Aflatoxin in a corn sample to review the procedure with students.

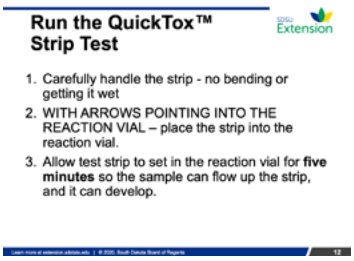
This can also be made available to students electronically or as a handout to refer to when conducting the experiment.

Slide	Notes
SDSU Extension **Procedure for QuickTox™ Kit for Aflatoxin Bulk Grain** Hands-on module for detecting the presence of aflatoxin in a corn sample. Food Safety Scientist Curriculum 	
Lab Safety Protocol SDSU Extension • Wear lab safety glasses, gloves (non latex) • Use caution when handling methanol and/or ethanol, they are both highly flammable and toxic. • Keep away from heat, sparks and flames. • Lab coat and exhaust hood is also advised for those preparing solvents.	
Aflatoxin in Corn SDSU Extension Problem/Issue • **Certain** molds that **can** grow on corn **may** produce aflatoxins. • Aflatoxins are harmful or fatal to livestock, and are considered carcinogenic to animals and humans. • Agricultural producers, elevators (storage), and food processing facilities continually monitor for the presence of molds and test for the presence of food toxins. • The FDA has acceptable aflatoxin levels in corn based on intended use: i.e., feed for dairy cattle – is zero	
***Aspergillus* mold** SDSU Extension Aspergillus on corn  Microscopic picture 	

How do you test for the presence of aflatoxin?
SDSU Extension
Three levels of testing
• Black Light test (also called the Ultraviolet light test)
  • Mold emits a greenish gold fluorescence under a black light.
• ELISA – immunoassay test kit
  • Detection of specific proteins found in aflatoxin
  • Uses antibodies to identify these proteins.
• Analytical Laboratories – use highly accurate tests to quantify the actual levels of aflatoxin.	

ELISA TEST – Enzyme-linked immunosorbent assay
SDSU Extension
• Four Steps:
  1. Prepare sample 2. Extract 3. Dilute 4. Run test
• SUPPLIES AND EQUIPMENT per test:
  • 1 or 2 test strips
  • Fixed volume transfer pipette (one for each test strip)
  • Reaction vial (one for each test strip)
  • corn sample – ground (20 mesh)
  • 70% methanol or 50% ethanol solution
  • plastic (or glass) sample container with tight fitting lid.
  • Graduated cylinder (10 or 25 ml)
  • Tap water
  • Timer
**items can change based on source of testing kit.*	

Slide	Notes
Interpret Results **Negative** Sample containing <20 ppb will develop 2 distinct lines in the test area (Control line and test line) **Positive** Sample contains ≥ 20 ppb will develop 1 distinct line in the test area (Control line) **Failed Test** No lines form in the testing area. Is this a quantitative or qualitative test? 	Is this a quantitative or qualitative test? This is a qualitative test – it tells us yes that aflatoxin is present above 20 ppb. It does not tell us the actually level. Quantitative tests are used for these purposes: thin layer chromotography, mass spectrometery,
Reference: This procedure was adapted from the QuickTox™ Kit for Aflatoxin Bulk Grain product insert, 12-27-06. This procedure is specific to this test kit. This is also available at the EviroLogix™ Company website: http://envirologix.com/artman/publish/index.shtml A step-by-step visual for using the QuickTox Kit for Aflatoxin can be viewed at http://www.envirologix.com/library/M68.pdf	
Obtaining Corn Sample 1. Ground Corn Sample – grind using food mill, Romer mill or coffee grinder (thoroughly cleaned and disinfect with 70% ethanol) 2. Measure out 10 grams of ground corn. 3. Place the 10 grams ground corn into the plastic sample cup with lid. Do you think it matters how finely the corn is ground?	Do you think it matters how finely the corn is ground? Yes it does. If you were doing this test for diagnostic purposes you would have all the equipment required to mill the corn to the size of a 20 mesh sieve. This ELISA kit has been developed for diagnostic purposes and it is important that good lab techniques are used so the results are reliable. If testing is not conducted with precision and accuracy, what could be the ramifications? 1) there could be a false positive and the corn would not be allowed to be used for food for humans or animal feed – this would be very costly, or there could be a lot of time and expense into further testing when it wasn't necessary. 2) A false negative would also be very serious – corn that is contaminated with aflatoxin would get into the food supply and put animals and humans at risk for serious health problems.
Extract aflatoxin from corn sample **Solubilizing aflatoxin, if present.** 1. Add two volumes of **solvent** (either 70% methanol or 50% ethanol) to the 10 grams of corn in the sample cup. Calculate the amount solvent needed _____ grams ground corn X 2 = _____ ml of solvent 2. Cap the sample cup tightly. Shake for 1 ½ to 2 minutes. The sample will separate into two layers, the top (yellowish) layer contains aflatoxin residues, if present. Why do we use ethanol or methanol as a solvent? 	Why do we use ethanol or methanol as a solvent? Aflatoxins are soluble in these solutions, and many of the other components of the corn are not. So, this will remove the aflatoxin from the corn into the liquid portion of the solution if it is present.
Dilute extract with water 1. Tap water (small amount in a very small sample cup) 2. Using the fixed volume pipette -150 microliters (µl), transfer tap water into the small reaction vial. 3. Use the same fixed volume pipette, transfer 150 µl from the top (yellowish) layer of sample. 4. Mix water and sample extract thoroughly by stirring with the tip of the fixed volume pipette	

Slide	Notes
	How does the sample move onto the testing area of the strip? Capillary action

Lab Report – Testing Ground Corn for Aflatoxin

Name:_____ Date: _____

Introduction:

What is the purpose of this lab?

_____

_____

_____

_____

Assume the ground corn sample is from corn that was slightly moldy and fluoresced (glowed) under a black light. What do you hypothesize the results will be, and why?

_____

_____

Results:

Fill in the table below:

Test Strip	Control Line Present (yes or no)	Test Line Present (yes or no)
Test Strip #1		
Test Strip #2		

Interpretation:

Is your sample positive, negative or a failed test?

_____

Discussion:

If the results are positive what does that mean?

_____

_____

_____

If the results are negative, what does that mean?

_____

_____

_____

What could cause a failed test?

_____

_____

_____

Is this a qualitative or quantitative test, and describe you answer?

_____

_____

_____

If you worked for a lab, and the results were negative, could you say the field of corn that your sample came from has no Aflatoxin present? Yes or no and explain your answer.

Were the results what you expected – yes, no and why?

Lab Report – Testing Ground Corn for Aflatoxin

Name:_____ Date: _____

Introduction:

What is the purpose of this lab?

To conduct a screening test on a sample of ground corn the presence of Aflatoxin in a corn sample. Aflatoxin can be fatal to animals, and is carcinogenic and cause other health problems for animals and humans. The FDA has very strict rules for keeping aflatoxin out of the food supply - <20 ppb in animal feed, and <0.5 ppb in milk (this is very close to zero tolerance).

Assume the ground corn sample is from corn that was slightly moldy and fluoresced (glowed) under a black light. What do you hypothesize the results will be, and why?

1. Positive because the corn kernels glowed when exposed to a black light.
2. The corn was moldy, so it is likely that Aflatoxin could be present.

Results:

Fill in the table below:

Test Strip	Control Line Present (yes or no)	Test Line Present (yes or no)
Test Strip #1		
Test Strip #2		

Interpretation:

Is your sample positive, negative or a failed test?

Discussion:

If the results are positive what does that mean?

Only the control line should have developed. The sample has at least 20ppb contamination level of Aflatoxin. More involved testing needs to be completed to identify the actual level of contaminate.

If the results are negative, what does that mean?

Both the control and test line developed. The level of contamination could be zero, or even less than 20 ppb. A test that tells actual levels needs to be completed.

What could cause a failed test?

A faulty test strip, or one that is past its expiration date.
Not following the lab protocol as directed – the QuickTox test strips may not been allowed to cool to room temperature.
The time for developing the test strip was not followed, among others.

Is this a qualitative or quantitative test, and describe you answer?

This is a qualitative test. It provides an answer that yes there is Aflatoxin present, above or below a certain level. It does not give the exact level.

If you worked for a lab, and the results were negative, could you say the field of corn that your sample came from has no Aflatoxin present? Yes or no and explain your answer.

No, you can only state that the sample was negative. One can extrapolate to the original lot or field that this was taken from only if the sampling procedure was followed correctly. Therefore, a disclaimer would be included with the test results referencing the following: These results are reflective only of the sample provided. This does not mean that the entire lot or field where the sample was obtained is entirely free of Aflatoxin at a detectable level.

Were the results what you expected – yes, no and why?

Answers may vary... need to reflect back to the hypothesis.

Reflection and Discussion Questions

Objectives: 1, 2, 5

What was being tested for in the corn? (Aflatoxin)

Further discussion: Imagine this: You open the cupboard and find that your bread is moldy. What do you do?

a. Encourage students to talk about mold on bread. What do they do with moldy bread? What conditions cause bread to mold?
   Moist, warm conditions, plastic bags.
   Maintain the warm, moist environment.

b. Why do we think mold is of concern?
   We've been taught to not eat moldy food. It has a bad smell. Molds are fungi; many fungi produce toxins that can cause illness.

c. Not all molds produce toxins. Can you think about fungi or mold that we eat on purpose, or they are beneficial for our health?
   Examples include the following: antibiotic penicillin (derived from Penicillium fungi); blue cheese (its blue veins are colonies of Penicillium fungi); huitlacoche (this corn fungus, Ustilago maydis, does not produce toxins and is eaten as a delicacy in Latin America); or even table mushrooms (Agaricus bisporus).

Guiding concept: Some molds are not harmful or are even helpful, while some are dangerous. Scientists help us identify which are dangerous, and can alert us when these dangerous kinds are on the food we may eat.

How was the black light used on corn mold? (as a presumptive test)

Where have we seen a black light (UV light)? What does black light cause to happen?

a. As students discuss the different colors and items that glow in a black-light, encourage them to understand that black-light makes some things seem more obvious, by emitting electromagnetic radiation in the near ultraviolet range. For example, store clerks can test bills for counterfeiting by passing them under a black light to test for fluorescent marks put there to authenticate the money. The marks are already there, but black light makes them more obvious.

b. How is a black light used in corn mold testing?
   Some fungi glow under black light.

c. What are the limitations of this way of testing?
   It is a presumptive test only for a mold that can produce a dangerous toxin —it tells you something is there, but not what it is. And, several different molds will fluoresce

d. What are the benefits of using the black light test?
   It's a simple, quick way to sort out corn that requires further testing.

Guiding concept: Black-light helps exaggerate things that we may not see with our own eyes. That is why it is a quick way to identify if corn may need to be held back for further testing before entering the food supply.

In the lab situation, why is it important to get accurate, reliable measurements?

Further discuss: When in science lab situations, it is essential to have accuracy and precision when measuring out the materials and conducting tests.

a. What types of measurements do you make in everyday life?

   Speed at which one drives, cooking units, temperature, air pressure, rain or snow fall. Students should be able to come up with a very extensive list.

b. How is measuring materials in the lab different from other measurements you make in daily life? For example, weighing your self in the bathroom, or measuring ingredients for baking?

   As students discuss measurements they have made, encourage them to see that it's hard to draw valid conclusions from inaccurate measurements.

c. Can you think of a circumstance where it would be important to know your own exact weight, or to get the proportions of ingredients in a recipe exactly right? How does this compare to what lab technicians do?

   In lab science the tests have to be very sensitive – often detecting in parts per million, billion (ppb) or even trillion. For example, consider an Olympic-size swimming pool holds about 500,000 liters (about 130,000 gallons) of water. One ppb would be one drop of a specific compound in the entire pool. In some instances lab tests have to be this specific. So, accuracy, precision and sensitivity of testing are very important.

d. When measuring the weight of a sample in a container, what simple step should you take to ensure that you're getting the right measurement?

   Measure the empty container first, and set the scale to zero (tare) before adding the sample. So you do not get the measurement of the container as well.

Guiding concept: Precise and accurate measurements are more important in a science lab situation. A test is accurate if it truly measures what it is suppose to. That is why science labs must always calibrate their equipment. Precision refers to the ability of the machine and scientist to repeatedly get the same results when measuring the same amount. It is important to be both accurate and precise for reliable and trustworthy results.

Interpreting the results of the test strip

Further discuss: Test strips are an easy and inexpensive way to test samples of corn for the presence of harmful molds and mycotoxins at an identified level. But you still need to use them carefully and correctly.

a. What are the markings on a test strip and what does each marking indicate?

   Test strips will vary: One line will often form to indicate that the test strip was working correctly. Additional lines will form to identify if the test is positive or negative for the presence of a specific component.

b. If the results were read or interpreted incorrectly by the person conducting the test, what would some of the ramifications be?

   False positive – identify the corn as possibly not safe and conduct unnecessary additional tests. Or destroy corn that is safe and needed by animals and humans.
   False negative – identify the corn as safe when it is not, and more testing should be completed. Distribute corn products into the food supply that may not be safe to consume.
   Ask students to compare this to a medical diagnostic test as well.

Guiding concept: A very important part of any test is knowing how to interpret the results.

Why is corn tested for mold? (for food safety)

Further discuss: We tend to take corn and other agricultural crops for granted. We assume that our food and other agricultural products will be plentiful, safe, and available.

a. What do you think would happen if corn wasn't regularly tested for mold?
   Toxins might be present and human and animal health would be at risk.

b. List some of the ways that corn is used in this country. Do you think you have consumed corn today?
   Used as livestock feed; also used in corn-based cereals, snack foods, sweeteners, sodas, corn oil, in medicines and cosmetics, and in ethanol as a component of auto fuel.

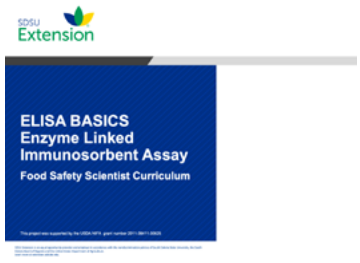
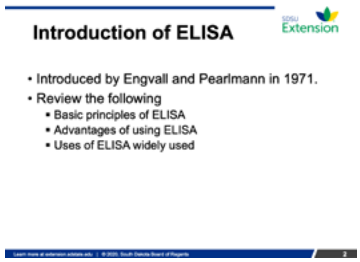
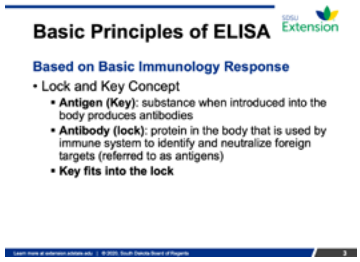
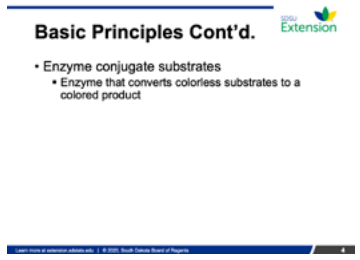
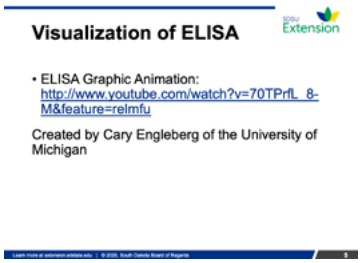
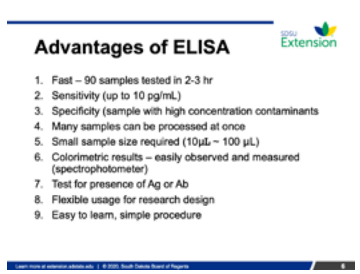
Guiding concept: Food safety testing impacts our health. Agricultural products like corn are present in many of the foods, drugs and many other by-products we encounter daily.

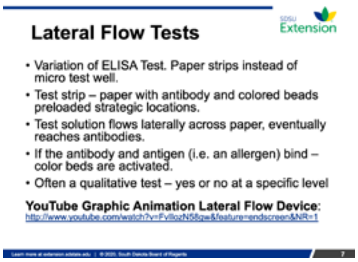
You've learned how to test a sample of corn for mold and Aflatoxin. Are the techniques you learned in this laboratory exercise only good for testing corn?

a. What are the basic, general skills you learned from the virtual lab?
   How to tare a scale and take precise and accurate measurements; how to use lab equipment such as a pipette and graduated cylinder; how to dilute a sample; and the importance of interpreting results correctly

b. What other lab work do you imagine might utilize the same skills?
   Soil science, water chemistry, medical and pharmaceutical testing, food science – testing for allergens in foods, many kinds of laboratory research science

Guiding concept: Basic laboratory skills learned in this corn mold lab could be the basis for employment in various fields.

Slide	Notes
	
	
	
	Enzyme conjugate substrate is either 1) Bound to the antibody that is part of the antibody-antigen complex (lock and key) Or 2) Bound to a secondary antibody that binds with the antibody-antigen complex
	This short animation demonstrates detection of specific antigens using the enzyme immunoassay. This resource was developed by Cary Engleberg of the University of Michigan. It is part of a larger learning module about laboratory methods for clinical microbiology (available at http://open.umich.edu/education/med/oernetwork/med/microbiology/clinical-micr...). 2009-2010, Cary Engleberg. This is licensed under a Creative Commons Attribution Noncommercial 3.0 License http://creativecommons.org/licenses/by-nc/3.0/.
	2. pg refers to picograms (one trillionth of a gram) 3. Since the testing can be done with high levels of contaminants, the sample does not need to be purified. 5. uL is a microleader - it is 1/1,000,000 of a liter 7. Ag – antigen and Ab – Antibody

Slide	Notes
	The lateral flow tests are usually qualitative. Using ELISA as described previously with the wells is a quantitative test.
	Ask for examples that they can think of for each Viral Contamination – blood for HIV and Hepatitis B and C Hormone levels – HCG – for pregnancy, LH for ovulation, thyroid function, anabolic steroids Infections – Sexually Transmitted Diseases – HIV, syphilis, chlamydia, hepatitis B and C, Toxoplasma gondii Specific disease factors – autoantibodies and autoimmune dieseases (rheumatoid factors and lupus) Drugs – illegal – amphetamine, opiates, cocaine, marijuana (active ingredient), performance enhancement Allergens in food – peanuts, tree nuts Residues in food – melanine, antibiotics Toxins in food – aflatoxin Others

Homework Assignment – Uses of ELISA and Lateral Flow Devices

Directions

Conduct an Internet search to find uses for ELISA testing and Lateral Flow test kits in the following areas:

- Diseases and or Infections
- Hormone levels
- Drug Residues
- Agriculture
- Specific food components
- Contaminants of food
- Other

Find at least one example of a specific ELISA or Lateral Flow Device test that is marketed on the Internet for the categories above (cannot use QuickTox™).

For each test identify the following:

1. Name of the test
2. Company that has developed and sold this test and what country it is located in.
3. Specific component that is being test for. For example, a specific toxin, GMO, allergen, heavy metal, antigen, antibody, among others.
4. Write these down to hand-in and be prepared to discuss in class.

Refer to at least one of the test kits you identified in your search and list all the types of careers that support this kit.