Food Safety Scientist

A Dynamic STEM Educational Adventure

Bacteria

The Good, The Bad and The Ugly

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ACKNOWLEDGEMENTS:

Curriculum:

Food Safety Scientist: A Dynamic STEM Educational Adventure
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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.

LAB SUPPLIES

Supplies needed for the virtual labs may be obtained from Educational Science Supply Companies. The creators of the curriculum, SDSU Extension, have kits available on a limited basis. Shipping and handling will be charged. To obtain supplies contact: joan.hegerfeld-baker@sdstate.edu.

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Contents of Unit

BACTERIA: THE GOOD, THE BAD AND THE UGLY

*Three Virtual Labs are a critical component of this unit:

1. Growing Bactieria- Inspecting a Dairy Processing Plant
2. Gram Staining (testing yogurt)
3. Oil Immersion Microscope

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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*
Virtual Lab-Disposable Lab Equipment	10 minutes	1,2,3,4,5,6,7,8	FCS 8, FCS 9.2,FCS 9.2.1, FCS 9.5.7, FS 2.2, MS-LS1-1, MS-LS1-5
Comparing Raw & Pasteurized Milk and Testing Surfaces	45 minutes to inoculate petrifilm & conduct swabs 30 minutes for observation and record results	1,2,3,4,5,7,8	FCS 8, FCS 8.2.1, FCS 9, FCS 9.2, FCS 9.2.1, FCS 9.5, FCS 9.5.7, AN6.1, FS 2.2, MS-LS1-1, MS-LS1-5
Video: Connecting the Dots-The Invention of 3M™ Petrifilm™ Plates.	5 minutes	1,2,3,6,7	FCS 9.5, FCS 9.5.7, AgP 1.3, FS 1.2, AS 1.1, MS-ETS1-2
Class Discussion	20 minutes	1,2,3,4,5,6,7,8	FCS 8, FCS 8.2.1,FCS 9, AgP 1.2,AgP 1.3, AgP 2.1, AN 6.1, FS 2.2, MS-LS1-1, MS-LS1-5
Virtual Lab-Gram Staining	10 minutes	1,2,3,4,5,7,8	FCS 9.2, FCS 9.5, FCS 9.5.7, FS 2.2., MS-LS1-1, MS-LS1-2
Hands on: Gram Staining	40 minutes	2,5	FCS 9.2 FCS 9.5, FCS 9.5.7, FS 2.2, MS-LS1-1, MS-LS1-2
Virtual Lab: Oil Immersion Microscope	10 minutes	2,3,4,5,7	FCS 9.2, FCS 9.5, FCS 9.5.7, FS 2.2, MS-LS1-1, MS-LS1-2
Hands on: Using a Microscope	15 minutes	2,3,4,5,7,8	FCS 9.2,FCS 9.5, FCS 9.5.7, FS 2.2, MS-LS1-1, MS-LS1-2
Discussion Guide: Gram Staining and Using a Microscope-Reflection & Discussion Questions	20 minutes	1,2,3,4,5,6,7,8	FCS 9.5, FCS 9.5.7, MS-LS1-1, MS-LS1-2

*See charts below 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 demonstrate laboratory science skills associated with growing bacteria, gram staining, and using a microscope.
6. Students will describe the various types of safe food handling practices and monitoring of the food supply.
7. Students will identify and give examples of various careers that support the safety of the food supply.
8. 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)

MS-LS1-1 – conduct an investigation to provide evidence that living things are made of cells; either one cell or many different numbers and types
MS-LS1-2- develop and use a model to describe the function of a cell as a whole and ways parts of cells contribute to the function
MS-LS1-5 – Construct a scientific explanation based on evidence for how environmental and genetic factors influence the growth of organisms
MS-ETS1-2 – Evaluate competing design solutions using a systematic process to determine how well they meet the criteria and constraints of the problem

GOOD BAD UGLY TEACHER RESOURCE SHEET

Materials Needed for "Comparing Raw vs Pasteurized Milk Lab" (Per lab group or student)

(4) 3M™Petrifilm™-Aerobic Count
(4) 3M™ Petrifilm™-E.Coli/coliform Count
(2) Disposable Pipettes
(1)3M™ Spreader
2 Tablespoons Raw Milk
2 Tablespoons Pasteurized Milk

Materials Needed for "Testing Surfaces for Microbial Contamination Lab" (Per lab group or student)

(8) 3M™ Quick Swabs
(1) 1 cm Swabbing Template
(4) 3M™Petrifilm™-Aerobic Count
(4) 3M™ Petrifilm™-E.Coli/coliform Count
(1) Spreader

Materials Needed for Gram Staining and Microscope Lab

Microscope (preferably with oil immersion lens)
Gram stain reagent set
Crystal violet
Gram's iodine
Decolorize reagent (95% alcohol)
Safranin

Background Information

- Before conducting this lab, read the Good Bad Ugly discussion guide. The discussion guide provides background information and many links to websites for additional information.
- To learn about the dangers of raw milk, visit: http://www.fda.gov/food/foodborneillnesscontaminants/buystoreservesafefood/ucm079516.htm#video
- To familiarize yourself with foodborne pathogens and illnesses visit: http://www.fda.gov/Food/FoodborneIllnessContaminants/default.htm
- To learn about homemade yogurt ("Good" bacteria) visit: http://www.uaf.edu/files/ces/publications-db/catalog/hec/FNH-00062.pdf
- To review proper Gram Staining techniques, visit: http://www.life.umd.edu/classroom/bsci424/LabMaterialsMethods/GramStain.htm

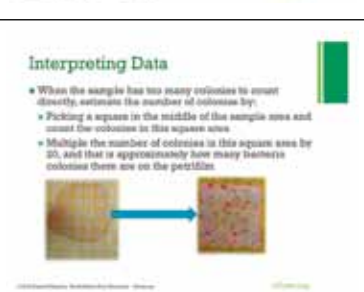
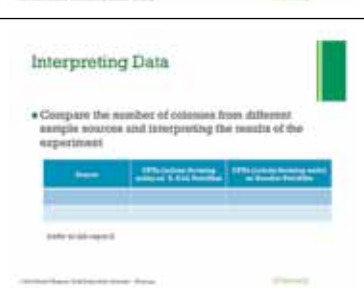
Other Notes

"Testing Surfaces for Microbial Contamination"

Students should sanitize their swabbing template with 70% ethanol sanitizing solution between each sample. Students should use paper towels or cotton balls to apply the solution. Each group should need approximately 4 ounces of solution total.

	
	Each group of students will need 10mL of raw and pasteurized milk.
	* Technically the students will only label 4 (2 of each) as the teacher will have the other two labeled as controls and incubated. Or, to show an example (other than this slide) the teacher could label one while presenting the powerpoint. And explain that they will be incubating the petrifilm that is selective for aerobic bacteria and E.coli bacteria.
	
	If an incubator is not available, a substitute incubator can be made. Place a paper towel in the base of an ice cream pail. Saturate the paper towel with tap water. Place a rack or items on the paper towel, (i.e. jar lids).Place the petrifilm on the improvised rack. Cover the pail. Set in a warm location. Using an improvised incubator may take longer than 48 hours for growth to become obvious.
	

LAB REPORT – COMPARING RAW VS. PASTEURIZED MILK AND TESTING SURFACE HYGIENE.

Name: _____

Date: _____

Introduction:

What is the purpose of this lab?

_____

_____

_____

_____

What do you hypothesize the results will indicate?

_____

_____

_____

_____

Results:

Raw vs Pasteurized Milk

1. Aerobic Petrifilm Plate Count Raw Milk Results:
   #1 = _____ Colonies / ml of milk
   #2 = _____ Colonies / ml of milk
   Average: _____ Colonies / ml of milk

2. Aerobic Petrifilm Plate Count Pasteurized Milk Results:
   #1 = _____ Colonies / ml of milk
   #2 = _____ Colonies / ml of milk
   Average: _____ Colonies / ml of milk

3. E. coli / Coliform Petrifilm Plate Raw Milk Count Results:
   #1 = _____ Colonies / ml of milk
   #2 = _____ Colonies / ml of milk
   Average: _____ Colonies / ml of milk

4. E. coli / Coliform Petrifilm Plate Pasteurized Milk Count Results:
   #1 = _____ Colonies/ml of milk
   #2 = _____ Colonies / ml of milk
   Average: _____ Colonies / ml of milk

5. Controls:
   Aerobic = _____ Colonies
   E. coli/Coliforms = _____ Colonies

Contact Surface Samples from Various Surfaces

1. Aerobic Petrifilm Plate count from Wet Swab
   Surface tested: _____
   #1 = _____ Colonies / cm2
   #2 = _____ Colonies / cm2
   Average:

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2. Aerobic Petrifilm Plate count from Dry Swab
   Surface tested: _____
   #1 = _____ Colonies / cm^2
   #2 = _____ Colonies / cm^2
   Average:

3. E. coli/Coliform Petrifilm Plate count from Wet Swab
   Surface tested: _____
   #1 = _____ Colonies/ cm^2
   #2 = _____ Colonies / cm^2
   Average:

4. E. coli/Coliform Petrifilm Plate count from Dry Swab
   Surface tested: _____
   #1 = _____ Colonies / cm^2
   #2 = _____ Colonies / cm^2
   Average:

5. Controls:
   Aerobic Petrifilm Plate:_____ Colonies/ cm^2
   E. coli/Coliform Petrifilm Plate:_____ Colonies/ cm^2

Discussion:

1. Why is milk pasteurized?

   _____
   _____
   _____

2. Why are food contact surfaces tested?

   _____
   _____
   _____

3. Why test for aerobic and E. coli/Coliform bacteria?

   _____
   _____
   _____

Interpretation/Conclusion:
From the Results what statements can you make that are conclusive? Were the results as you expected?

_____
_____
_____
_____

LAB REPORT – COMPARING RAW VS. PASTEURIZED MILK AND TESTING SURFACE HYGIENE.

Name: Teacher Version

Date: _____

Introduction:

What is the purpose of this lab?

Compare the level of microbial contamination of raw milk to pasteurized milk. The types of bacteria investigated are coliforms and aerobic bacteria. The testing of various surfaces will give an indication of the level of contamination surfaces. For example, this information can be used to compare surfaces before and after they were clean and/or sanitized.

What do you hypothesize the results will indicate?

No coliforms will be present in the pasteurized milk. The level of contamination of aerobic bacteria will be much higher in the raw milk, compared to the pasteurized. Surfaces that have a lot of contact by people (i.e. computer key board) will have higher levels of contamination. Surfaces that have been cleaned and/or sanitized will have a lower level of contamination.

Results:

Raw vs Pasteurized Milk

1. Aerobic Petrifilm Plate Count Raw Milk Results:
   #1 = _____ Colonies / ml of milk
   #2 = _____ Colonies / ml of milk
   Average: _____ Colonies / ml of milk

2. Aerobic Petrifilm Plate Count Pasteurized Milk Results:
   #1 = _____ Colonies / ml of milk
   #2 = _____ Colonies / ml of milk
   Average: _____ Colonies / ml of milk

3. E. coli / Coliform Petrifilm Plate Raw Milk Count Results:
   #1 = _____ Colonies / ml of milk
   #2 = _____ Colonies / ml of milk
   Average: _____ Colonies / ml of milk

4. E. coli / Coliform Petrifilm Plate Pasteurized Milk Count Results:
   #1 = _____ Colonies/ml of milk
   #2 = _____ Colonies / ml of milk
   Average: _____ Colonies / ml of milk

5. Controls:
   Aerobic = _____ Colonies
   E. coli/Coliforms = _____ Colonies

Contact Surface Samples from Various Surfaces

1. Aerobic Petrifilm Plate count from Wet Swab
   Surface tested: _____
   #1 = _____ Colonies / cm^2
   #2 = _____ Colonies / cm^2
   Average:

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2. Aerobic Petrifilm Plate count from Dry Swab
   Surface tested: _____
   #1 = _____ Colonies / cm^2
   #2 = _____ Colonies / cm^2
   Average:

3. E. coli/Coliform Petrifilm Plate count from Wet Swab
   Surface tested: _____
   #1 = _____ Colonies/ cm^2
   #2 = _____ Colonies / cm^2
   Average:

4. E. coli/Coliform Petrifilm Plate count from Dry Swab
   Surface tested: _____
   #1 = _____ Colonies / cm^2
   #2 = _____ Colonies / cm^2
   Average:

5. Controls:
   Aerobic Petrifilm Plate:_____ Colonies/ cm^2
   E. coli/Coliform Petrifilm Plate:_____ Colonies/ cm^2

Discussion:

1. Why is milk pasteurized?
   To destroy pathogens that are commonly associated with raw milk. Such as *Listeria Monocytogenes*, Salmonella, Campylobacter, and E.coli.

2. Why are food contact surfaces tested?
   To identify if food contact surfaces have been adequately cleaned and sanitized to stop the spread of microorgansims (i.e. raw juices from poultry contaminating a cutting board that could be used for cooked or ready-to-eat food products.)

3. Why test for aerobic and E. coli/Coliform bacteria?
   These microorganisms are often associated with foodborne illness, and they can be an indication of fecal contamination

Interpretation/Conclusion:

From the Results what statements can you make that are conclusive? Were the results as you expected?

REFLECTION AND DISCUSSION QUESTIONS:

Virtual Labs:

- Growing bacteria (inspecting a processing plant, aka disposable lab)
- Gram Staining (testing yogurt)

Introduction:

Good bacteria and bad bacteria – that is often a criteria that people will use to categorize bacteria. However, bacteria and other microorganisms found in food, such as fungi (yeast, and molds) are considered pathogenic (Bad), spoilage (Ugly) and in some instances beneficial (Good). Microorganisms can also be harmless…just hangin' out.

Below are two guided discussions to serve as a review of the Virtual Lab: Growing Bacteria with disposable supplies and equipment. The purpose of this guide is to build upon some basic concepts and applications that are related to food microbiology and food safety.

1. Beneficial Microorganisms (related to food)
2. Pathogenic Foodborne Microorganisms

Beneficial microorganisms (related to food):

Guiding concept: Bacteria and are often suspected to be harmful to humans – particularly in our food. However, the human body needs bacteria that are beneficial such as those that improve our intestinal health. Some bacteria and fungi (yeasts and molds) are useful in processing and preserving foods. Learning how to use the microscope in testing bacteria in a yogurt sample is the first step to identify beneficial bacteria from their bad relatives.

Food Processing and Fermentation:

Yogurt is produced by fermenting milk with specific bacteria. Fermentation of food is commonly used to process food for making alcoholic beverages, leavening of bread and preserving foods.

1. What is food fermentation?
   a. Fermentation used in the food and beverage industry to convert carbohydrates into alcohol, carbon dioxide and organic acids (i.e. lactic acid and acetic acid). The fermentation process often takes place in an anaerobic environment – which means no oxygen is present.

2. In the Gram staining virtual lab yogurt was being analyzed to see if undesirable bacteria were present.
   a. Yogurt is a fermented food. What food product is fermented to make yogurt? What are the microorganisms that are used in yogurt making? Can you make yogurt in your home?
      - Yogurt is made from milk – it is best to use milk that has been pasteurized. Why is that important? – destroy pathogenic organisms (mostly gram –) and at the same time some spoilage organisms are destroyed. The sugar in milk (lactose) is utilized by the bacteria to produce an acid. The acid that is produced lowers the pH of the milk causing proteins to form a gel resulting in the consistency of yogurt.
      - The two common bacteria used to make yogurt are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*
      - Yogurt can be easily made in the home using basic kitchen utensils. There are several good recipes on the Internet that are easy to follow. Always use a pasteurized milk product to reduce the risk of foodborne illnesses commonly associated with the consumption of raw milk.

3. Fermenting milk to make yogurt is also a form of preserving milk to extend the shelf-life. Processing milk made into yogurt extends the shelf life for several weeks.

4. What other foods are preserved by using a fermentation process?
   a. Fermented vegetables – sauerkraut, Kimchi, certain pickles. These are often made by adding salt to change the environment to promote the growth of lactic acid producing bacteria. The bacteria utilize the carbohydrates (sugars) in the vegetables to produce lactic acid giving the product a sour taste.
   b. Dry sausages such as salami – sugars are added to ground meat that lactic acid producing bacteria feed on. This lowers the pH, therefore proteins will denature and coagulate. This also lowers its ability to hold water – creating a dried food product that has been preserved for longer storage time. The process takes many weeks (several months).
   c. Beverages of wine, beer and cider.
      - Wine is made from yeast that utilize the sugars of the fruit juices to produce alcohol.
      - Beer is made from yeast that utilizes the starches from cereal grains to produce alcohol.
   d. Cider is a made from the fermentation of apple juice by yeast to produce alcohol. This is often referred to as hard cider because of the alcohol content.
   e. Leavened breads – yeasts utilize the sugar from the starches of cereal grains to produce gas – causing the bread to rise.

Probiotic Bacteria are beneficial to the human body by improving the balance of bacteria in the intestine.

1. How can bacteria in our intestine be beneficial to us?
   a. Inhibiting pathogens and toxin producing bacteria from reproducing in our intestine
   b. Several specific health effects from probiotic bacteria are being investigated and documented that address chronic diseases.

2. How do we get probiotics in our diet?
   Some probiotics are added to food products to improve the products' health benefits for consumers, yogurt is the best example. The probiotics commonly added into yogurt include bacteria from the Lactobacillus Family (LAB).

Pathogenic Foodborne Microorganisms

Underlying Concept: The Centers for Disease Control estimates that each year roughly 1 in 6 Americans (48,000,000 people) get sick, 128,000 are hospitalized, and 3,000 die of foodborne diseases. (1)

Leading Foodborne Illnesses (FBI):

1. What is a foodborne pathogen?
   a. A disease causing microorganisms commonly transmitted or found in a food sources

2. What is a foodborne illness?
   a. A FBI is an illness that develops from consuming a food that was contaminated with a disease causing agent.

3. What is a FBI outbreak? And, can you think of any foodborne illness outbreaks that have occurred?
   a. A FBI outbreak is when two or more people get the same disease from eating the same contaminated food. For example, two people develop Salmonellosis from drinking the same source of contaminated unpasteurized milk. They must have the exact same strain of Salmonella. And the same strain must be in the food source as well.
   b. Several examples of foodborne illness outbreaks in the United States (and in some cases internationally):
      - Shiga-toxin producing E. coli O104 in Germany – 784 patients with hemolytic uremic syndrome (HUS)–a type of kidney failure, 23 deaths associated with HUS. Four confirmed cases in the United States. http://www.cdc.gov/ecoli/2011/ecoliO104/index.html#introduction
      - Investigation Announcement: Multistate Outbreak of Human Salmonella Typhimurium Infections Associated with Exposure to Clinical and Teaching Microbiology Laboratories - As of April 20, 2011, a total of 73 individuals infected with the outbreak strain of Salmonella Typhimurium have been reported from 35 states. http://www.cdc.gov/salmonella/typhimurium-laboratory/042711/index.html
      - Botulism associated with commercially canned chili sauce – Texas and Indiana, July 2007. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm56d730a1.htm?s_cid=mm56d730a1_e
      - Additional Foodborne Illness outbreaks can be reviewed at the Centers for Disease Control Outbreak Response team's website: http://www.cdc.gov/outbreaknet/

4. What is pasteurization and why is milk pasteurized?
   a. Pasteurization destroys pathogenic microorganisms by heating the milk very quickly to destroy pathogenic microorganisms, then cooling it very fast.
   b. Why is the milk heated and cooled so quickly?
      - The milk first needs to get to a temperature that is high enough to kill the pathogens that are commonly associated with raw (unpasteurized) milk.
      - This process occurs very quickly to keep the milk from getting a cooked flavor. Underlying Concept: The Centers for Disease Control estimates that each year roughly 1 in 6 Americans (48,000,000 people) get sick, 128,000 are hospitalized, and 3,000 die of foodborne diseases. (1)
      - The cooling process also happens very fast, once again so it doesn't develop a cooked flavor, and to obtain refrigeration temperatures so bacteria that are still present in the milk do not grow.

5. Does pasteurization destroy all the microorganisms in the milk?
   a. No – it destroys mostly pathogenic organisms. If all the microorganisms were destroyed that would be sterilizing the milk.
   b. Unlike sterilization, pasteurization is not intended to kill all pathogenic microorganisms in the food or liquid. Instead, pasteurization aims to reduce the number of viable pathogens so they are unlikely to cause disease (assuming the pasteurization product is refrigerated and consumed before its expiration date). Commercial-scale sterilization of food is not common because it adversely affects the taste and quality of the product.

6. Do you think very many people get sick from drinking unpasteurized milk, or eating products made from unpasteurized milk?
   a. Not like the number of people that get the common cold. The FBIs that are often a result of unpasteurized milk are very serious, and people can get very ill. (Unlike the common cold).
   b. From 1998 through 2008, 86 outbreaks due to consumption of raw milk or raw milk products were reported to Centers for Disease Control. These resulted in 1,676 illnesses, 191 hospitalizations, and 2 deaths. Because not all cases of foodborne illness are recognized and reported, the actual number of illnesses associated with raw milk likely is greater. (2)

To learn more about the risks of consuming unpasteurized milk, visit the Centers for Disease Control Website. http://www.cdc.gov/foodsafety/rawmilk/raw-milk-index.html. This website is very comprehensive and includes videos of families and individuals that become seriously ill from consuming unpasteurized milk.

(1) CDC. (2011). "2011 Estimates of Foodborne Illness inte h United States." Retrieved 6-17-11, 2011, from http://www.cdc.gov/Features/dsfoodborneEstimates.

(2) CDC. (2011). "Raw Milk Questions and Answers." Retreived 6-27-11, from http://www.cdc.gov/foodsafety/rawmilk/raw-milk-questions-and-answers.html

SDSU Extension Lab Protocol: Gram Staining Yogurt Sample or Milk (raw or pasteurized)	

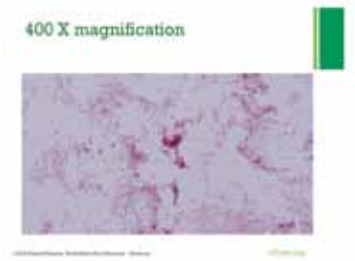
Equipment
• Inoculating loop
• Bunsen Burner
• Clean microscope slide – clean
• Pipette (disposable)
• Clasp (clothes pin, or tongs)
• Gram stain reagent set
  ◦ Crystal violet
  ◦ Gram's iodine
  ◦ Decolorizer reagent (95% alcohol)
  ◦ Safranin
• Microscope (preferably with oil immersion lens)	

Gram Staining – View Bacteria • Gram staining process stains bacteria. The color can be observed under the microscope. • Bacteria that stain a pink or reddish color are called **Gram-negative.** • Bacteria that stain a purple color are called **Gram-positive.**	
Procedure – Gram Staining **Prepare work area:** • Wipe down the lab table with 70% ethanol solution. • Turn on the gas valve and use the striker to light the Bunsen burner.	
Procedure **Preparing Slide:** 1. Draw a circle in the center of a glass slide with a permanent marker. 2. The circle indicates where the small sample of yogurt will be placed. 3. Turn the slide over so the markings and the sample will be on the opposite sides. 4. Label the slide with date, sample source, and personal initial in the labeling area.	
Procedure **Sample dilution and inoculation:** • Transfer 1 milliliter (mL) of water (distilled or deionized) into a test tube. • Thoroughly mix your sample (yogurt, milk, other food sample) with a spoon. • Sterilize the inoculation loop in the flame of the Bunsen burner. *(Hold it directly in the flame until it glows red hot.)*	

	
	Lets identify and list the function or purpose of each part of the microscope: (this is an animated slide) 1. Eyepiece – focus and magnify the object (usually 10X) 2. Objectives – the lens that focuses on the object. Magnify – the lens in this picture is probably 40X 3. Nosepiece – houses the objectives and is used to rotate the objectives and secure them in place for focusing. 4. Stage – holds the specimen you are studying 5. Stage Clip – secures the slide that you are studying 6. Coarse focus – use this first to get the specimen (bacteria) you are studying in view. 7. Fine Focus – use this after the coarse focus, or when going to a higher power to get a detailed image in focus. 8. Arm – use to carry the microscope. 9. Diaphragm – controls the amount of light that shines onto the specimen. 10. Light source – shines a light on the image so the lens can then focus.
	
	

	Gram Stained Slide… Need a Microscope to see the individual bacteria from the specimen on this slide.
	Rotate the nosepiece to the lowest magnification, 10x. Keep rotating the nosepiece until you hear the "click" sound, which means the lens is at the right position to use. Place the previous prepared slide on the stage of the microscope and secure it with the clips. Use the diaphragm to adjust the amount of light that goes through the slide.
	When doing this demonstrate with the microscope you have in the classroom. Some microscopes have a 4x and some may have a 10X With some microscope the nosepiece moves up and down – not the stage. Bring the objective lens (10x) as close to the slide as possible. 1. Focus the image by adjusting two knobs (coarse and fine adjustments) of the microscope. 2. Start with the coarse adjustment and rotate it to bring the stage as close to the lens as possible. 3. Slowly rotate the coarse adjustment to bring the sample into focus. 4. Once you see a vague image, very carefully rotate the fine adjustment knob to make the image as sharp as possible. 5. You may need to move the slide so the image is located where it can be focused upon.
	In some instances you may have to slightly adjust the coarse focus. Usually not. 1. Rotate the nosepiece to the 40x lens. (difficult to see in this photo 2. Only use the fine adjustment to bring the image into focus. 3. Once again, adjust (move) the slide to position the image if needed. 4. For the next observation, you are going to use the oil immersion lens, which needs to be used with a small amount of immersion oil.

	1. Rotate the nosepiece halfway between the 40x and 100x (oil immersion objective lens). 2. Use the applicator adding one drops of immersion oil on the slide – lighted area. 3. Finish rotating the oil immersion objective lens into place. 4. The oil immersion lens should be just touching the drop of oil 5. Extremely carefully and slowly rotate the fine adjustment to bring the image into focus, and avoid rotating the fine adjustment too fast to break the slide or lens. 6. Now the bacteria should be clear enough for observation. Observe the color of bacteria and discuss the result of observation. Why use Immersion oil? Keeps the light from refracting or bending so it can be used to focus the image. Do not return back to a lower magnification lens after this step.
	Image of bacteria from a sample. Notice the different shapes some are called cocci (round) Bacilli (rods) This is a magnification of 1000x (100x objective lense and a 10x Eye-piece lens)
	
	

	What does that mean? Answer: the image appears 100x larger that what it really is.
	Ask: What does it mean to autoclave something? Placing it in a chamber with extremely high heat to sterilize it (destroy all bugs – viruses, bacteria, parasites). This is used in research labs and hospitals.