Kansas Corn:

Hitting the Bullseye – Accuracy and Precision in Laboratory Pipetting

This lab is made possible with the support and content contributions of the Kansas Corn Commission.

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Kansas Corn: Accuracy and Precision in Pipetting

Grade Level: High School

Overview

Any educator electing to perform demonstrations is expected to follow *NSTA Minimum Safety Practices and Regulations for Demonstrations, Experiments, and Workshops*, which are available at http://static.nsta.org/pdfs/MinimumSafetyPracticesAndRegulations.pdf, as well as all school policies and rules and all state and federal laws, regulations, codes and professional standards. Educators are aunder a duty of care to make laboratories and demonstrations in and out of the classroom as safe as possible. If in doubt, do not perform the demonstrations.

The corn industry has made major advances in the last thirty years. Many of these advancements have come on the heels of an increased incorporation of biotechnology into the seed development sector. Since 1996, when the first herbicide resistant varieties were brought into production, many of the most important advancements have developed thanks to countless hours spent in laboratories developing and cultivating new varieties. The skills we need to develop in the next generations of these scientists must be discretely taught. There is no better way to do that than through hands-on laboratory experiences. Simply put, there is no substitution for putting the tools in the hands of the learner. In this investigation, students will have an opportunity to develop skills relative to volumetric measurement on a very small scale. Students will be using adjustable volumetric pipettes to accurately measure small amounts of liquids, as precisely as possible, using authentic tools that can be found in 21st century labs across the country and the world. Students will also see a crossover between the development of lab skills and the art of lab work by precisely depositing specific volumes in order to create patterns or pictures of their own design.

Kansas College and Career Ready Standards Addressed:

Science and Engineering Practices from Next Generation Science Standards

- Analyzing data in 9–12 builds on K–8 experiences and progresses to introducing more detailed statistical analysis, the comparison of data sets for consistency, and the use of models to generate and analyze data.
  - Analyze data using tools, technologies, and/or models (e.g., computational, mathematical) in order to make valid and reliable scientific claims or determine an optimal design solution.
  - Consider limitations of data analysis (e.g., measurement error, sample selection) when analyzing and interpreting data.
  - Analyze data to identify design features or characteristics of the components of a proposed process or system to optimize it relative to criteria for success.

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Kansas College and Career Ready Standards Addressed (Continued):

Science and Engineering Practices from Next Generation Science Standards (Continued)

- Mathematical and computational thinking in 9-12 builds on K-8 and experiences and progresses to using algebraic thinking and analysis, a range of linear and nonlinear functions including trigonometric functions, exponentials and logarithms, and computational tools for statistical analysis to analyze, represent, and model data. Simple computational simulations are created and used based on mathematical models of basic
    - Apply ratios, rates, percentages, and unit conversions in the context of complicated measurement problems involving quantities with derived or compound units (such as mg/mL, kg/m3, acre-feet, etc.).

Math

- **N.Q.1. (all)** Use units as a way to understand problems and to guide the solution of multi-step problems; choose and interpret units consistently in formulas; choose and interpret the scale and the origin in graphs and data displays.
- **N.Q.2. (all)** Define appropriate quantities for the purpose of descriptive modeling.
- **N.Q.3. (all)** Choose a level of accuracy appropriate to limitations on measurement when reporting quantities.
- **F.IF.1.(all)** For a function that models a relationship between two quantities, interpret key features of expressions, graphs and tables in terms of the quantities, and sketch graphs showing key features given a description of the relationship. Key features include: intercepts; intervals where the function is increasing, decreasing, positive, or negative; relative maximums and minimums; symmetries; end behavior; and periodicity.
- **F.LQE.1. 11)** Construct exponential functions, given a graph, a description of a relationship, or two input-output pairs (include reading these from a table).
- **S.ID.1. (9/10)** Use statistics appropriate to the shape of the data distribution to compare center (median, mean) and spread (interquartile range, standard deviation) of two or more different data sets. **(S.ID.2)**
- **S.ID.2. (9/10)** Interpret differences in shape, center, and spread in the context of the data sets using dot plots, histograms, and box plots, accounting for possible effects of extreme data points (outliers). **(S.ID.3)**

Art

- **VA:Cr2.1.I** Engage in making a work of art or design without having a preconceived plan.
- **VA: Cr1.2.II** Choose from a range of materials and methods of traditional and contemporary artistic practices to plan works of art and design.
- **VA:Cr2.1.II** Through experimentation, practice, and persistence, demonstrate acquisition of skills and knowledge in a chosen art form

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Learning Objectives:
- Students will discover uses for basic laboratory equipment found in professional lab settings.
- Students will accurately operate a micropipette with precision sample placement.
- Students will assess their own skills using a pipette by calculating percent error and standard deviations of a body of sample data.
- Students will understand the variety of agriculture-related jobs where basic laboratory skills are required to be successful.

Materials Needed:
- Pipette Technique PowerPoint (available online at www.kscorn.com)
- Hitting the Bullseye Student Sheet (pg. S1, or available online at: kscorn.com)
- Waxed paper or Parafilm
- Printed grids (1/5 inch graph paper can be printed for free from the following website: bit.ly/PipettingGridPaper)
- Micropipette (with 20-200 ul volume ability)
- Micropipette tips (non-sterile)
- Small clear plastic cups
- Digital scale
- Food coloring
- Computer (with access to the internet or Microsoft Excel)

Safety Considerations:
- **Be aware of students with sensitivity to certain artificial dyes. Using food grade dyes (liquid food coloring) eliminates most safety concerns.**

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Procedures for Instruction

Length of Time for Preparation: 10 min
Length of Time for Classroom Teaching: 30-45 minutes or longer depending on your preference

Preparation Procedure/Instructions:

- Students will need an approximately 4-inch square of waxed paper, doubled. Cut into approximately an 8 x 4-inch rectangle and then fold in half so two thicknesses can be used.
  - Note: One thickness of Parafilm in a 4-inch square can also be used if available.
- Prepare colored water samples.
  - Note: If using food coloring, simply combine 20-30 ml of water and a few drops of food coloring in a small plastic cup. Most liquid food coloring comes in red, blue, yellow, and green, which is more than sufficient to complete this lab.
- Make enough cups of food coloring so each student has access to a cup. Make sure they are easily accessible as spilling of the cups may occur.
- Setting up the station: Each station should have access to a micropipette and "sample liquid." If possible each station would be best served with a scale as well. Access to a computer with Microsoft Excel software for data analysis will also be important.

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Classroom Discussion: Introduce the topic and assess students for prior understanding:

Use *Hitting the Bullseye Student Sheet* (pg. S1, or available online at: kscorn.com)
Discussion of Accuracy and Precision:

- Have students work in pairs to define the term "accuracy" or "accurate". What does it mean to be accurate? Brainstormed ideas can be written on the T-Chart of the Hitting the Bullseye Student Sheet (pg. S1, or available online at: kscorn.com).
- Go over the responses together as a class and come up with one simple definition of accuracy. Write that definition on the board and make sure students write this on their Hitting the Bullseye Student Sheet.
- Repeat the same questioning process and collaborative discussion for the term "precision".
- Have students provide a visual representation of each definition by leading them through the following: On your graphic organizer, draw what it means to be "precise" and "accurate" on the appropriate picture. If you are a sharp shooter, and you are very accurate, what would your bullseye look like? Show at least 5 shots. Repeat the process with "precision". If you are a sharp shooter, and you are very precise, what would your bullseye look like? Show at least 5 shots.
- Pose the questions:
  - Is it possible to be accurate and precise?
  - Accurate and imprecise?
  - What about precise but inaccurate?
  - How would that look if you were the sharp shooter from before? (Make sure you draw these marks in pencil so that corrections can be made.)
- As a class go over the final graph organizer and show how each bullseye would look differently in each condition.
- Why is this important for science research? Have students discuss the question, you can give the guiding prompts.
  - What if two scientists come up with different data for the same research? How do we know who is correct? How would we check?
  - What if these two scientists are trying to complete a lab that requires microscopes because the sample is so small, would we want them to use large instruments to complete the lab or smaller ones?
- What is our goal in science? Accurate and precise – we want our data to be repeatable and be correct! So how do we measure this? What kinds of things can affect our accuracy and our precision?
- For further clarification and a great video explanation on the concepts of accuracy and precision visit Mr. Evan's Science site at the following link:
  - https://sites.google.com/a/apaches.k12.in.us/mr-evans-science-website/accuracy-vs-precision

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Procedure for Lab:

Part 1: Overall pipette function, Volume selection and use: Technique and accuracy.
15-20 minutes

Pipette Technique PowerPoint (available online at www.kscorn.com)

- Note: The PowerPoint includes photos of proper use and technique for students to reference.
- Making sure you have good technique when using appropriate tools is one way you can increase your accuracy. The same way you would shoot free throws in practice to ensure you are ready for the big game, in lab work you want to make sure your skills are polished and ready! Practice, practice, practice…
- Have students practice the soft and hard stops on the micropipette. Set the volume to 25 ul and practice, paying special attention to how far the students must move their thumb to reach the soft and hard stop. Now move to the 50 ul volume and repeat. Note the difference in the distance your thumb must move in order to draw up and expel the different volumes.
- Set pipette to 25 ul, practice drawing up a sample. Depress to the soft stop, place tip of the pipette into the solution, being careful not to touch the sides or the bottom of the container, release your thumb allowing the plunger to move back up, drawing the solution into the pipette tip. Note the amount of liquid in the tip, and the absence of any air bubbles.
- Over the waxed paper, expel the sample. Depress the plunger all the way to the hard stop. Hold the hard stop as you bring your hand and the tip of the pipette above the small sphere of fluid on the waxed paper before releasing the plunger. If you release the plunger before removing the tip from the fluid, you will draw the fluid back into the pipette.
- Repeat this process multiple times so you can become comfortable with the proper technique. If you find you have air bubbles in the bottom of the pipette tip, expel the sample back into the cup and try again. Make sure you are using proper technique!
- Change the volume to 50 ul and repeat the process above in an area at least 2 squares away from your first sphere. Note the difference in the size of the sphere relative to your first sample. This sphere should be approximately twice as large since you are selecting to use twice the volume.
- Repeat these steps as many times as needed to become comfortable with proper technique.
- Using your pipette combine two spheres of sample, one 25 ul and one 50 ul, by placing the tip of the pipette into one sphere and carefully dragging the sphere toward the other sample until the two spheres merge.

- Adjust the volume of the pipet to 75 ul, try to draw up all of the sample in this now merged sphere. If you accurately used your pipette with proper technique, you should be able to take in all of the sample without having bubbles at the end of your pipette tip.
- Continue to practice this skill, changing volume on the pipette and combining bubbles to draw up larger samples until you feel sufficiently comfortable.
  - NOTE: This is also a possible lab practicum assessment possibility, have students repeat this process in your process to ensure the proper technique is being use.

Part 2: The right tool for the job?
20-30 minutes

Scenario: You are in a lab setting with very limited access to lab equipment. You have been tasked with the job of pipetting 2 ml of sample onto a surface. You only have two tools to use, a micropipette, with variable volume selections, and a scale. Your job is to figure out what setting to use in order to pipette the 2 ml of sample with both accuracy and precision.

- **Step 1:** What should your volume setting be?
  - You know that your pipette can choose sample between 20 and 200 ul. You know that 1000 ul = 1 ml so…. How many ul would you need to measure total? (1000 ul (2 ml) = 2000 ul)
  - How can you measure that total using this one instrument?
    - Possible options:
      - 10 samples of 200 ul
      - 20 samples of 100 ul
      - 16 samples of 125 ul
      - 40 samples of 50 ul
      - 80 samples of 25 ul
  - You decide that you will test 3 sample sizes to determine the precision of your equipment. (Note: Not all equipment is precise at all volume.)

- **Step 2:** You have no other way to check your overall measurement in terms of volume but you know 1 ml of water = 1g, so you can use the scale!!!
  - Your total value should be 2 g of sample when weighed using the scale. (Note: the scale has been recently calibrated and you know for certain is quite precise and accurate.)
  - Place the waxed paper onto the scale, tare or zero your scale if it is registering any weight with only the waxed paper.
  - Now pipette the appropriate number of samples onto the waxed paper. As a good scientist you are going to take this opportunity to make sure your technique is flawless! So you will work to make sure you are being precise with your sample placement by not allowing the sample bubbles to touch one another.
  - Note the weight in g for this trial of your data.

- **Step 3:** Calculate your accuracy using the % Error formula. This is one way to use a mathematical model to see how far away you are from "the bullseye".
  - %Error = 100 (measured weight – expected weight) / expected weight
  - The smaller % Error the better!
  - Compare your % errors for each of the sample volumes you completed in Step 2. Make an assessment about which volume selection you feel you can pipette with the most accuracy.

- **Step 4:** Check your precision or % CV (Coefficient of Variation). This is one way to use a mathematical model to see how far away your shots are from one another.
  - Using the volume you felt would be the best suite in this case, check your pipette's precision. We now have to see if you have repeatable data!
  - Repeat the process of Step 2, 10 more times, remembering to record the overall weight at the end of each trial.
    - This should not take more than 2 minutes per trial when choosing the larger volumes.
    - If using waxed paper make sure you are using a new piece of waxed paper for each trial so you are not inadvertently creating error with water that has soaked into the paper.
  - Use the data you collected to complete a statistical analysis.
    - Input the trial measurements into a Microsoft Excel file in one column.
    - At the bottom of the column type in the following formula:
      - =STDEV(A2:A13)/AVERAGE(A2:A13)*100
      - Adjust the cell tags if necessary.
      - You are simply using Microsoft Excel's short cut for determining the standard deviation for a set of data. This is an example of a mathematical model that we can use to assess our data.
      - The closer your calculated value is to a 0 value, the more precise you are in your measurements.
  - Repeat this process with the other volumes you selected from Step 2.

PART 3: Analysis and Decision time:
20-30 minutes

- Using your mathematical models determine what is the optimal volume setting for this particular task.
- Why would having a variable pipette be a good thing? How does it affect your overall % Error if you are completing less samples for one total volume?
- What are some things that could affect your overall accuracy in this lab?
  - How could you control these variables?
- What are some things that could affect your overall precision in this lab?

    - How could you control these variables?
- Why is it important for scientists to ensure their tools are correctly calibrated?
- In major seed development labs, where precision and accuracy can be vitally important, most pipetting is automated and controlled via computer. How would this increase the accuracy and precision of their labs? How would that impact the quality of their overall product?

Note: Some students have difficulties steadying their hand when using pipettes. As precision in sample placement is part of proper technique, it is important to go slow and provide plenty of time for student to practice before any lab practicums or skills assessments. One possible aid to students is to place both elbows on the table while grasping the pipette in both hands during use. This technique allows for more stability by providing support for the arms and wrists.

Video and student examples available at kscorn.com.

Reflection and Conclusion:
During these activities students will have developed some basic laboratory skills that will allow them to experience, on a very cursory level, the act of doing science precisely, while maintaining good technique for accuracy. As with all research, repeatable data is one of the major keys to success, and without proper technique and precise equipment that goal is not achievable. Whether using a wrench, screwdriver, or pipette, there is always a right tool for the job. This lab allows students to develop those skills, while also providing them with an experience to see how some simple techniques will allow them to become marketable in the biotech field, as well as other types of labs around the country and the world.

Assessments:
As a possible lab practicum have students show you each step as they go through the process of depositing two samples of different volumes, combining samples, and changing the volume of the pipette to withdraw the whole combined sample, with one sample draw, as outlined in Part 1. This shows proper technique and is one step closer to using them in an actual lab setting.

Using mathematical models to draw conclusions is a major part of research. It is not only important to know how to do the work and collect the data, but also to analyze the data in determining what the data means. Students must understand how mathematical models are utilized as tools just as any other equipment in the lab. Numbers don't tell us much; it is what those number represent that provide us with meaningful information we can use to draw conclusions.

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Science and Agriculture Careers

Biotechnology and agriculture have been working hand in hand since humans started domesticating plants and animals. Humans have worked towards creating and finding specific breeds and lines of organisms in order to reach the ultimate goal of meeting the growing needs of humanity. In the 21st century, lab work and skills are very much a part of that solution. Jobs in fields such as biofuel development, food safety and product development, micro plant propagation, seed development, molecular biology, pathology, biochemistry, geneticists, entomologists, agronomists, and many others will be the key to the future of agriculture. Agriculture needs highly skilled individuals, with solid backgrounds in laboratory practices and practical work, if they are going to fill the positions that are needed to solve the problems of producing and protecting our resources and ourselves.

Note: To learn more about agriculture careers visit agexplorer.com. You can also find career profiles at kscorn.com.

Sources:

- Graph paper – http://www.printfreegraphpaper.com/gp/c-i-15.pdf

NOTES:

Hitting the Bullseye

Accuracy	Precision
	

Low Accuracy, Low Precision 	Low Accuracy, High Precision 
High Accuracy, Low Precision 	High Accuracy, High Precision