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PROFESSOR: Many laboratory procedures, especially biological and biochemical ones, require the precise transfer of small quantities of liquids. Glass pipettes, which are commonly used in chemistry labs, are simply not accurate when working with volumes that are less than 1 milliliter. The best method of transferring small volumes of liquids involves the use of an automatic pipette.

An automatic pipette consists of a piston that displaces air to create a vacuum when its plunger is pressed. The tip of the automatic pipette is then placed into the sample vial and the plunger is slowly released. This action draws liquid into the tip. Finally, this liquid is expelled into the desired container by slowly depressing the plunger a second time.

As shown, a variety of automatic pipettes exist. This video will demonstrate automatic pipetting technique using the Pipetman. Using good technique will ensure accuracy, which is defined as the closeness of the dispensed volume to the set volume, and precision, which is defined as the reproducibility of individual measurements between your samples.

A Pipetman consists of a plunger button, a tip ejector button, a volume adjustment knob, a volume indicator, and a plastic shaft. Pipetman are available in six different sizes. Five of them are depicted here.

The number on the plunger button indicates the maximum volume in microliters that each Pipetman is capable of transferring. For example, the P20 has a possible volume range of 0 to 20 microliters. However, transferring volumes at the low end of the range is not recommended because the standard deviation is too high to ensure precision. Thus the minimum amount of liquid that can be transferred in microliters is 0.1 for the P2, 0.5 for the P10, two for the P20, 10 for the P100, 50 for the P200, and 100 microliters for the P1000.

Now that we've covered what an automatic pipette is and how it works, we'll take a detailed look into how the Pipetman is used in the laboratory. Let's say, for example, your procedure requires you to transfer 153.5 microliters of sample. The first step involves the selection of the Pipetman with an appropriate volume range.

In this example, a couple of choices exist. The P200 has a recommended volume range of 50 to 200 microliters while the P1000 has a recommended range of 100 to 1,000 microliters. Remember the lower end of a volume range always has a higher standard deviation. Therefore the P200 is the better choice.

The second step is the selection of the proper disposable plastic tip. Three tip sizes exist-- the large blue tips, which fit the P1000, the medium yellow tips, which fit the P200, P100, and the small white tips, which fit the P10 and P2. Because we're using the P200, we're going to need the yellow tips. Using the proper tip is essential for preventing leaks and promoting accurate delivery of liquids.

After selecting the correct Pipetman end tip, set the desired transfer volume. For the smaller Pipetman, P2 to P200, the volume set on the indicator translates exactly into the volume that will be delivered. This P200 is set to deliver a volume of 125.9 microliters. For the P1000, the volume set on the indicator must be multiplied by 10 to determine the actual volume that will be transferred. This P1000 is set to deliver a volume of 1,259 microliters.

To correctly adjust the volume setting, turn the volume adjustment knob to one third of a revolution past the desired volume. Then slowly move the dial back down to the desired setting. Always approach the desired volume by dialing downwards to prevent mechanical backlash and to ensure precise volume transfer. When adjusting the volume of a Pipetman, take care not to rotate the dial past the maximum. You cannot transfer volumes above the range of the Pipetman.

Now it's time to transfer your sample. You will need your appropriately sized Pipetman set to the desired volume, the correctly sized tips, a rack, the new container, a chem wipe, and a waste container. Attach a disposable tip to the Pipetman by pressing its plastic shaft firmly into the tip with a twisting motion. This action should ensure an airtight seal and prevent leaks from occurring. Always use a new sterile tip for each sample and never let the tip touch anything except sample.

To draw a sample into the plastic tip, depress the plunger until resistance is felt. Hold the Pipetman vertically and immerse it into the sample. After the tip is immersed, slowly release the plunger to draw liquid into the tip. Wait a few seconds to ensure that the full volume of sample is drawn into the tip. As a guide, wait one second for the P2 to P200 Pipetman and 2 to 3 seconds for the P1000. The larger the volume or the more viscous the sample, the longer you should wait.

Remove the Pipetman from the sample and carefully wipe excess liquids from the sides of the tip. To dispense the liquid in the Pipetman, touch its tip to the side wall of a new container and slowly depress the plunger to the first stop. Wait a few seconds to ensure complete sample flow, then press the plunger to the second step to expel any remaining liquid.

With the plunger fully depressed, carefully remove the tip from the container by sliding it up the container wall. The importance of sliding the table along the wall increases as the volumes become smaller. Finally, allow the plunger to return to the up position. If you are done working with a particular sample, press the tip ejector button to release the tip into the proper waste receptacle.

Several other guidelines will help to ensure accuracy and precision when transferring liquids. First, use a fresh tip for each sample to avoid contamination.

Second, pre-rinse each new tip. Most liquids leave a film on the inside wall of a new tip, which affects the actual volume that can be transferred. By pre-rinsing a tip, these volume variances are eliminated. To pre-rinse a tip, simply draw solution into the tip and expel it into a waste container.

Third, check the immersion depth. If the immersion depth is too deep, liquid may get into the plastic shaft and damage the mechanics of the Pipetman. If the immersion depth is too shallow, air may be drawn into the tip, which greatly affects accuracy.

As a general rule, immerse the white tips to a depth of 1 to 2 milliliters, the yellow tips to a depth of 2 to 3 milliliters, and the blue tips to a depth of 2 to 4 millimeters. If you do find an air bubble in the tip, dispense the sample into a waste container, check your immersion level, and pipette more slowly. If you still see an air bubble, discard your current tip and use a new one.

Fourth, release the plunger button slowly. Do not let the plunger snap up. This violent action may force liquid into the plastic shaft. Fifth, when wiping excess liquid off the sides of the tip, avoid touching the tip opening because some of the sample may be lost. Six. When liquid is in its tip, never allow the Pipetman to tilt more than 20 degrees from vertical and never lay the Pipetman on its side.

For optimum reproducibility, it is important to be consistent with your samples. Specifically, use a consistent speed and smoothness when pressing and releasing the push button. Use consistent pressure at the first step. Immerse the tip to a consistent depth. And hold the pipetman at a consistent angle that is less than 20 degrees from vertical.

If you are confident that you used good technique and are still seeing errors with accuracy or precision, the following troubleshooting guidelines may fix your problem. To ensure that your Pipetman is actually delivering the desired volume, you should periodically calibrate it.

To calibrate a Pipetman, determine how many grams of your liquid equals 1 milliliter from its density. For example, if water is the solvent, then 1 milliliter of water equals 1 gram of water. Tare a small container on an analytical balance and set the Pipetman at a volume near the top of its range. Transfer the set volume of liquid to the tared container on the balance and note the mass of the liquid.

Repeat this step five more times and determine the mean weight and standard deviation from your measurements. Repeat the entire process with the Pipetman set at a volume near the bottom of its range.

If these values deviate from what you expect, adjust the pipetman accordingly when you transfer liquid. Occasionally, the Pipetman will begin to leak due to improperly fitted tips or worn o-rings. To check for leaks, put a fresh tip on the Pipetman. Pre-rinse the tip and draw up liquid. Keep the Pipetman vertical and wait about 20 seconds. If you see a droplet, then the Pipetman is leaking.

Two final things that decrease the accuracy and precision of the Pipetman involve pipetting samples below 4 or above 70 degrees Celsius and using the Pipetman with acids and corrosives. If you absolutely must pipette an acid or corrosive, then you should disassemble the Pipetman immediately to inspect and clean the piston, shaft, seal assemblies, and o-rings.

To summarize, using proper technique with an automatic pipette is essential for ensuring accuracy and precision. A few key points to remember include the following. Be consistent with plunger timing and pressure as well as immersion time and depth. Use a fresh, pre-rinsed tip for each new sample. Check for air bubbles and leaks during each transfer. And calibrate the automatic pipette periodically.

In addition, take care not to drop the expensive and easily damaged Pipetman. Do not allow the tip to touch anything except sample. Do not introduce liquid into the plastic shaft by letting the plunger snap up or by laying the Pipetman on its side.

Finally, avoid using the Pipetman under extreme temperature conditions or with acids and corrosives. When used with consistency and care, an automatic pipette is an excellent means of transferring microliter quantities of liquid.

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