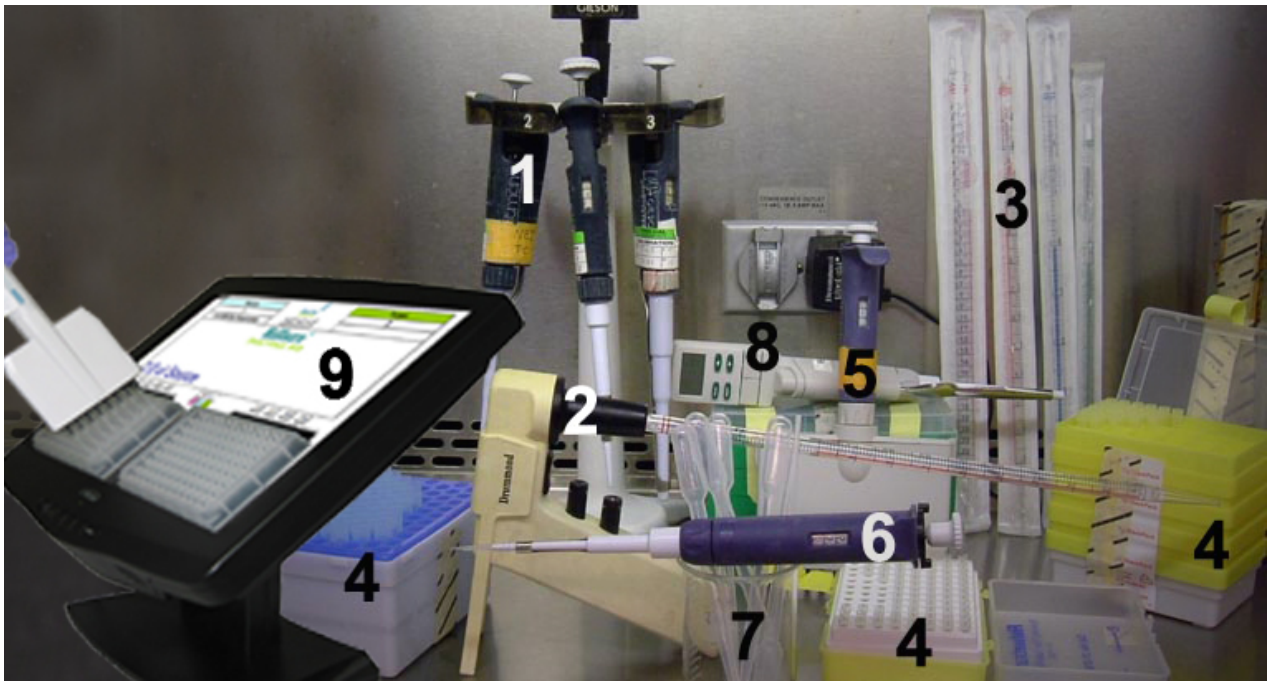
COC: 'Introduction to Biotechnology" Custom Lab Exercises



Volumetric Techniques: Theory and Practice: Serological and Micropipettes

Version 6-29-12

- In biotechnology, there are a range of metric measurements that must be made. Previous labs have introduced you to the typical range of metric units, conversions, etc.
- Of the metric units, volume is perhaps the most crucial as most of biotechnology (and biology) is water (or solution) based.
- Solutions can be measured and transferred with a range of "volumetric glassware" including: flasks, beakers, graduated cylinders and pipets.
- Small volumes and expensive reagents make pipets the most commonly seen devices in a biotechnology laboratory. This lab will clarify the roles of serological and micropipette in biotechnology by focusing on techniques designed to improve technique of both serological and micropipettes.
- Subsequent lab activities will focus of choosing what pipet to choose and the impact this decision has on the accuracy of the volume transferred.

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1. Objectives:

1. Introduce students to the concept of volume transfer and use of micropipettes and serological pipettes.
2. Refine theory and technique associated with using serological pipettes by practicing volume transfer using both mechanical and electrical pumps.
3. Understand effect of serological pipette volume on accuracy by calculating and comparing errors associated with transfer.
4. Review micropipette general technique and practice.
5. Refine micropipette technique and verify pipette accuracy and precision using a variety of exercises designed to clarify both the theory and practice of micropipette use.

2. Background:

There is perhaps no greater icon of biotechnology than the pipette. This device invokes up images of technology and is found in science (and biotechnology) labs around the world. There are range of pipettes and applications, from simple transfer pipettes and disposable pipettes made of plastic, to multi-channel pipettes that allow for automated devices to transfer small volumes with incredible accuracy and precision. The range of pipettes can be partially viewed by looking at the coversheet. There are a number of pipettes and pipette parts shown. If you have a minute, revisit the cover and try to identify the items show (reflect on the first lab, where you were provided the names of a number of these devices). The key to this activity is at the end of the lab, and if the image is poor, try using the on-line version of the lab to better view these devices. This lab will introduce you to serological and micropipettes. These two types of devices are used in almost every biotechnology lab application and thus, a fundamental working awareness of these apparatus is essential. Lastly, pipet or pipette are both correct spellings FYI.

Using Serological Pipettes.

Serological pipets are made of glass or plastic. Glass ones are rare and are often saved an autoclaved to conserve the resource. Typical volumes range from 1-25 mls with lots of specialized versions available. The pipet is used in concert with a pump of some type as pipetting by mouth is a cardinal sin! While the pipets are sterile and or autoclaved, the pumps are usually sterilized with a simple alcohol wipe. The range from simple hand pumps (color coded to help identify different sizes) to electric hand held devices and automated, multichannel gizmo associated with high trough put automation.

A. Volumetric Determination and Error

During many procedures, scientists and technicians often must transfer a given amount of solution from one container to another. Accuracy and time management are often essential, so sophisticated machines have been developed to facilitate quick and accurate transfer. The accuracy of the pipette is related to the size of its smallest unit of measure. Transfer pipettes are made of plastic, and have the bulb fused to the column. The column usually has demarcations on it that allow for measurement as low as 10 µl (although they are often not as accurate as serological pipettes.) A micropipette is a calibrated device that can measure liquid down to fractions of a microliter and typically has a range from 1 ml to 0.01 µl. When first learning to work with a serological pipette, take some time to familiarize yourself with the following pointers.

1. Take a few minutes to note the range of volumes, various sizes of BOTH the pipets and the pumps.

2. If the technique involves sterility issues, always remove the pipet from the container, sheath, etc. by pressing it into the pipet pump and try to avoid touching it. FYI, today's activity does not observe sterile technique, so the pipets are probably not wrapped.
3. Take a few minutes to note the scale. They come in variety of forms that can tell the user how much the aspirated (sucked up), or expelled (pushed out). Some pipets have both scales. So, DO NOT naively assume that the pipet volume is obvious. Take a minute to review the scale and its implications.
4. For SAFETY sake, be careful when pushing pipet into pump. Hold the pipet near the end that goes into the pump. Holding it too far away could snap the pipet and drive the broken pipet into your hand!
5. As you work with the pipet scale, this rule will change, but when you are just getting used to the pipet, ONLY ASPIRATE WHAT YOU INTEND TO DELIVER!

For the following exercise you will be using a serological pipette.

Use a 5 ml serological pipette and a 100 ml beaker.

Before you begin, weigh the empty beaker and record your results below.

Now take the 5 ml pipette and transfer the following volumes of water to the beaker:
5.0, 4.5, 4.3, 4.2, 4.0, 3.5, 3.3, 3.2, 3.0, 2.0, 1.5, 1.0, 0.5 ml

These volumes should be transferred to the beaker individually and remember BOTH points 3 and 5 above as they can really impact the volume! The total volume of the 13 combined volumes should be 40 ml. How close you came to the theoretical result can be defined on the basis of the total weight of the water in your beaker. The density of water at 23° C (room temperature) is 0.9976 gm/ml.

B. Use dimensional analysis to determine the theoretical weight of 40 mls of water.

Weight of beaker and water	=	_____
Weight of empty beaker	=	_____
Weight of water (experimental)	=	_____
Calculated weight of water (theoretical)	=	_____
Deviation from expected	=	_____

To determine the amount of your error (your deviation, as expressed as a percent of the expected value), you *subtract the expected or theoretical weight of the water from the weight of the water that you actually transferred (experimental value).* This first step gives you information as to how much weight your sample was over or under (deviation from) the expected weight. To find how acceptable this deviation is, you simply *divide the value above by the theoretical value* to find out what fraction of the expected your deviation represented. Normally error is expressed in percent and to convert your value to a percent, simply multiply by 100. While this sounds confusing, the equation below illustrates the calculation:

$$\% \text{ Error} = \frac{\text{Experimental value} - \text{theoretical value}}{\text{Theoretical value}} \times 100\%$$

Here is the above equation expressed in terms of our specific experiment

$$\% \text{ Error} = \frac{\text{Measured wt. of } H_2O - \text{calculated wt. of } H_2O}{\text{Calculated wt. of } \% H_2O} \times 100\%$$

% Error from your experiment _____

A deviation of 1-2 % is not unusual. <u>If 5 % is seen, practice your technique again.</u> Note the percent error is expressed as an absolute number, (no negative percent please!)

How would you explain the error in your experiment? List at least three possible causes.

1.

2.

3.

Once you have mastered volumetric transfer with the 5 ml pipette. Repeat the above process with 25 ml 10 and 1 ml pipettes. Please note: for the purpose of the serological pipettes, you can use the mechanical pipette aids. If you want to, you can also try out the electric pipette pumps. If you choose to try these devices, make sure you try all three transfers with the same device. Do not do one transfer with a mechanical pipette pump and two with a electric, etc. Record your information below:

Pipette volume: 1 ml: The numbers should be less by a factor of ten for this exercise, so…0.5 ml, 0.45 ml, 0.43 ml, etc… and weighs 4 grams?

Trial One:	Trial Two:	Trial Three:
Weight of beaker and Water _____	Weight of beaker and Water _____	Weight of beaker and Water _____
Weight of beaker ____	Weight of beaker ____	Weight of beaker ____
Weight of water, exp. ____	Weight of water, exp. ____	Weight of water, exp. ____
Weight of water, calc. ____	Weight of water, calc. ____	Weight of water, calc. ____
Deviation _____	Deviation _____	Deviation _____

Pipette volume: 10 ml (use volumes from 5 ml SOP)

5.0, 4.5, 4.3, 4.2, 4.0, 3.5, 3.3, 3.2, 3.0, 2.0, 1.5, 1.0, 0.5 ml

Trial One:	Trial Two:	Trial Three:
Weight of beaker and Water _____	Weight of beaker and Water _____	Weight of beaker and Water _____
Weight of beaker ____	Weight of beaker ____	Weight of beaker ____
Weight of water, exp. ____	Weight of water, exp. ____	Weight of water, exp. ____
Weight of water, calc. ____	Weight of water, calc. ____	Weight of water, calc. ____
Deviation _____	Deviation _____	Deviation _____

Pipette volume: 25 ml: (use volumes from 5 ml SOP)

5.0, 4.5, 4.3, 4.2, 4.0, 3.5, 3.3, 3.2, 3.0, 2.0, 1.5, 1.0, 0.5 ml

Trial One:	**Trial Two:**	**Trial Three:**
Weight of beaker and Water _____	**Weight of beaker and Water _____**	**Weight of beaker and Water _____**
Weight of beaker ____	**Weight of beaker ____**	**Weight of beaker ____**
Weight of water, exp. ____	**Weight of water, exp. ____**	**Weight of water, exp. ____**
Weight of water, calc. ____	**Weight of water, calc. ____**	**Weight of water, calc. ____**
Deviation _____	**Deviation _____**	**Deviation _____**

Show the above tables to the instructor when finished.

Micropipette Practice: (NOTE: Read this portion of the lab completely prior to starting work with the micropipette and delegate accordingly).

GENERAL TECHNIQUE OVERVIEW….

Getting familiar with the micropipette:

A. The pipette used in this experiment come in three different ranges: 2-20 ul, 20-200 ul and 100-1000ul. The volumes measured are very small since there are one million microliters in a liter.

B. Try to visualize these volumes (you will note that this is difficult as these are very small volumes, and on the pipette scale at first glance they can look identical.) Note the diagram listed (of the scale panel for a 2-20 µl pipet) below to become familiar with the approximate volumes as they appear on the pipette.

NOTE…(*XXXX*) If the pipette were to be forced to this volume (0.2 ul) it would destroy the device (and this is clearly outside of the 2-20 ul range for the device)

20.0ul ---→	**2**	2.6ul---→	**0**	**0.2 ul →**	**0**
	0		**2**	**ones**	**0**
Red decimal line					
	0		**6**	**tenth**	**2XX!**
				Broken!	
				Out of range	

Become familiar with the function of the volume adjuster (the black dial in the handle of the device, just below the colored button you depress to operate the device.)

- With the number display facing you:
- --------> increases volume
- <-------- decreases volume

Memorize these "pipette nevers"

- Always check the pipet range on the top of the colored button at the top of the handle (yellow, blue or white / grey).
- Never rotate volume adjuster below 2 or above 20 microliters (or outside the range, or the pipette may jam.)
- Never use without a tip in place.
- Never lay the pipette down with fluid in it.
- Never let the pipette button (the one with the range printed on it) snap back.

Become familiar with how to add and eject a tip.

Insert pipette tip into disposable tip and twist to snugly fit in place. Press the ejector button to remove the tip. Try this until you are very comfortable with the process.

- Loading and emptying: The yellow button has two stops. The first stop is for filling and dispensing the sample. The second stop is to "blow out" the last little bit of the sample. Find these stops and make sure you are very familiar with these stops.

Loading:

- Depress button to first stop. Dip pipette tip into sample and draw fluid up by gradually releasing button. Make sure you keep the tip submerged in the fluid during this step.
- Remove the pipette tip from the sample and examine the sample. It should extend all the way to the pipette tip. There should be no air at the tip or anywhere along the length of the fluid.
- Make certain that you understand the importance of depressing the button to the first stop for filling. If you depress to the second stop, you may get 5 or more extra microliters of sample (often a catastrophic amount!)

B. Emptying:

- Take a clean microfuge tube and touch the pipette tip to the bottom of the microfuge tube.
- Depress the button to the first stop to expel the sample.
- Depress the button to the second stop to "blow out." Try stirring the sample while the button is still depressed (to aid in delivery.)
- Keep the button down while removing the pipette from the sample
- Gently release the button.

Micropipette Practice Exercises:

The following 6 exercises are all designed to challenge your technique in some specific way. Micropipetting is a basic skill in any biology lab and it is VERY important you PRACTICE these activities. Do not read, do once and say…OK what next? On the other hand, we do not have time for you to take hours for each activity…***So, try each one activity at least once and preferably, two or more times***. After going through all of the activities (and reviewing comments, results from the rest of the class) then you should return to those activities that you feel you need the most work on? Lastly, do not change tips unless really necessary. For practice activities, the tips can be reused unless damaged or changing between solutions of different make-up.

Exercise One: "The 2,3,5 exercise".

This exercise helps refine technique and also helps to calibrate your pipette! If after doing the exercise a few times, you STILL get weird results, it may suggest your pipette is out of whack (let the professor know). See other exercises for additional insights into pipette accuracy and precision.

1. Using a colored practice solution (1.5 ml microfuge vials: labeled: R,G,B,Y) pipette the following amounts into a clean tube with a 2-20 ul pipette: 5 microliters, 3 microliters, 2 microliters If needed, spin the sample in a balanced centrifuge to ensure all of the sample is at the bottom.

2. To check that your measurements were accurate, set the pipette to 10 microliters and withdraw all the solution. Is the tip filled all the way to the end? There should be no residue left in the tube. If there is fluid left in the tube, or if there is an air space in the tip, your technique could use some improvement.

Note: The idea of a 2ul, 3ul, 5ul experiment works something like this. Assume you pipette is off by 0.1 ul . If you aspirate 2.0 ul according to the dial, the device actually pipettes 2.1 ul. So the volumes transferred will be 2.1, 3.1 and 5.1 ul. This volume will then equal 10.3 transferred. When you set the pipette to 10 ul, it will aspirate 10.1, and reveal a 0.2 ul volume error! So…if while doing this exercise and all of the following pipette exercises, DO NOT HESISTATE to talk with me if you suspect your pipette is not working. If after three trials, you still do not get ideal results, it may be that your pipette is indeed damaged. Keep this idea in mind when you do the last exercise in this series (#6). This exercise will allow you to compare your pipette to a whole bunch of other pipettes, and hopefully highlight any problems if they exist.

5 hints for general accuracy:

1. **Be patient**. Wait a few second after aspirating. Slowly transfer. Slowly expel.
2. **Always hold the pipette in the vertical position, with the tip down**. This helps with accuracy and avoids the solution getting inside the shaft of the pipette itself!
3. **Watch the sample**: Note when it enters the tip and enters the final location. Do not assume some "got in", especially with small volumes and clear solutions. Look at the tip to ensure the solution got in. Also deliver the solution to the side of the microfuge tube to see the sample getting delivered. Spin the sample to mix as needed.
4. **Take special care** with cold samples, viscous samples or samples with solids in them. Heating and centrifuging may help make the solution more "transfer friendly".
5. **Use correct pipette**. Consider 20 ul. Both a 2-20ul and a 20-200 ul pipette can transfer this volume, but the 2-20 is the better choice. Generally, accuracy is greatest at the high end of the device…so 2-20 is a better choice for 20 ul than a 20-200 ul pipette is.

Exercise Two: "2,3,5 exercise on steroids".

For the following pipettes, try 2,3,5 exercise with noted volumes and use the R,G,B,Y vials.
2-20 ul pipette:
Put in: 1.7 ul, 3.4 ul and 4.9 ul, take out 10 ul.
Put in 12.3, 3.4 and 4.3 ul, take out 20 ul.
Put in 2.0 ul, ten times…. take out 20 ul….

20-200 ul pipette:
Put in 23 ul, 127 ul and 50 ul take out 200 ul.
Put in 145 ul, 26 ul and 29 ul take out 200 ul.
Put in 20 ul, 10 times and take out 200 ul.

100-1000 ul pipette:
Put in 165 µl, 610ul and 225 ul take out 1000ul.
Put in 334 ul, 121 ul and 545 ul take out 1000 ul.
Put is 100 ul, ten times, take out 1000 ul.

Exercise Three. Volume assessment: "Drops".

To help you develop a intrinsic sense of small volumes, please complete the following exercises in pairs. Once each member of the team has completed a series, make the comparison as noted.

NOTE: The small pieces of wax paper easily blow away (NOOOOOOOOO!..so be carefull)…and you will want to be careful with the dye. It will stain, but washes out with regular detergent and is just harmless food coloring FYI..

Take 8 pieces of Parafilm (a wax paper / plastic used for sealing containers) and tape (just the very edge of the paper, leaving plenty of room to work with) onto a larger piece of paper of notebook paper. This will make it easier to handle the individual squares. Next to each square of wax paper, write one of the noted volumes: 2 µl,5 µl, 10 µl, 50 µl 100 µl, 500 µl and 1000 µl: . Onto these separate pieces of wax paper, place 2 µl,5 µl, 10 µl, 50 µl 100 µl, 500 µl and 1000 µl of a similar colored solution using appropriate pipettes. Transfer the entire amount in one load and try to make it a single drop. IF YOU GET MULTIPLE DROPS, do not worry. You can push the drops together using the pipette tip, thanks to hydrophobic, hydrophilic expulsion! Place side by side and compare volumes visually.

Now, working in pairs, have one person pick up the individual squares (be careful as you peel them free to not dislodge the droplet) and show them to each the other person. Can you visually ID the volume? Now switch off. Try placing smaller volumes side by side (3 different volumes) and see if it is easier than seeing the squares one at a time to determine the volume.

Once you have complete the square exercise, SAVE YOUR DROPS for the next experiment.

Exercise Four: Volume Assessment: Microfuge tubes:

Next, get one of the PCR tube strips and repeat the above sequence. If you want to be frugal, use the old droplet to get the volume you need! Provided it has not been too long (and the drops have not evaporated much) you should have just the right volume. Check the pipette tip to see if it is full? If it is NOT full, then get some dye from the original tube and work with this.

Add the volumes, left to right into the PCR tube strip: 2 µl,5 µl, 10 µl, 50 µl 100 µl, 500 µl and 1000 µl.

NOTE: Depending on the PCR tubes and the other resources provided, you may not be able to complete the series, as the tubes only hold so much volume or there are only 6 tubes?

Using a pair of scissors (or brute force if you prefer), pull the tubes apart and try the volume comparison exercise with your lab partner (hold up the tubes and ask each other what is the volume.)

Once you have completed exercises 3 and 4, take a minute to clean up the lab. All of the waste is garbage friendly FYI. Be sure to save your colored dye tubes (the "stock" tube with all of the liquid (R,G,B,Y) , not the PCR tubes) as you will need them later, so place them back in the appropriate rack.

Exercise Five: Chasing the sample.

Sometimes a large sample is pulled from a very full microfuge tube. If you were to simply insert the pipette tip into the solution, the microfuge tube would overflow! So…you have a dilemma. You can solve this by learning the following technique. We will repeat it twice to see the effects of viscosity on pipetting samples.

Exercise Five A: Chasing the sample, low viscosity:

To complete this exercise, get a microfuge tube and fill it to the brim using the 1000 ul pipette. Depending on what microfuge tubes you are provided, you may need to add 1, 1.5 or 2 mls. Get the colored water from one of the 50 ml falcon tubes (AKA centrifuge tubes) and get the amount you need. Please return the tube when done collecting your sample. Once the microfuge tube is full, set the pipette to 1000 ul and try to get a full load out of the tube without spilling any. To do with, you will need to closely watch the tip and have careful thumb control.
Depress the pipette to the first stop.
Insert the tip just barely under the surface of the solution. Be careful to watch for overflow (and prevent it).
As you release the tip…SLOWLY… you will notice the solution level in the microfuge tube is dropping.
As this is happening, you will need to push the pipette tip further and further into the sample, again taking care to not cause overflow.
As you release the thumb, you will be pushing the tip into the solution, making sure you always have the tip under the solution. This way, you are effectively "chasing the sample".
Double check the volume transferred by inspecting the tip. Is it full to the tip? Has any of the solution spilled out? Is the amount remaining in the tube what you expect it to be?
Practice this activity until you are clear on both the technique behind it and the rational for it.

Exercise Five B : Chasing the sample, viscous solution.

Repeat the basic experiment outlined in 5A, except this time, start with the very viscous solution. These are in special 50 ml Falcon tubes labeled viscous or glycerin: R,G,B,Y. A viscous solution is one that resist the ability to flow. Water flows relatively readily, where are honey flows less so. So, honey is more viscous than water.

As you complete this exercise, please be aware of two things:
1. Note the effect the viscosity has on accuracy and technique.

2. Be more aware of where this solution ends up. It is essentially a colored, dyed sugar syrup and as a result, it can get REALLY STICKY. Please remember to clean up every spill and to exercise caution so that *none of this stuff gets in or on the pipette*.

Exercise 6. Accuracy verses precision: Filter paper SOP:

This lab addresses the idea of accuracy verse precision. Assuming that your pipettes up to now have been working OK, then this final test will OK them for lab work. This test looks at the idea of accuracy verses precision. Hopefully by now, your technique is getting better (although your thumb may be getting tired).

NOTE: The following SOP may use different sized filter paper. The size of the filter paper may affect the size of the drops you can add to the paper. TO get an idea as to how far to place the drops (especially the larger drops) please note the image on the last page of this SOP. It shows the drops smearing together (in the larger volumes) so…note the scale bar (ruler) and place your drops an appropriately large amount apart.

Also…this lab exercise can be VERY MESSY! The dye will bleed through the filter paper (so place some paper towels under the filter paper). T he larger volumes tend to saturate the filter paper while the sample is being added. The effect is to create a small volume of liquid that may "burp" when any air is introduced. This volume of air is often associated with the end of the delivery stroke on the pipet, when the operator is trying to get the last bit of liquid out by depressing the pipet to the second stop. In a nutshell…think ahead and be prepared to some leaking, splashing and smearing…. In the end, it is just food coloring, but it can make a mess.

Please remember: The following exercise results will be compared to others in the class. Be sure to save all of your data and to label everything clearly. A result of reviewing this data, we will decide to possibly calibrate and/or fix a pipette. So, do not just post your results, remember which pipette gave you which results (perhaps a small piece of tape, etc to help you remember the pipet may be a wise idea).

Using a piece of provided filter paper and colored solution. Be sure to note the paper and solutions provided by the professor, as this REALLY affects the results. Obviously we all need to use the same paper and same samples dye if we are going to compare results.

Label the filter paper in the lower right corner with your name. Also note the pipette number or any other identifying information (volume, type, etc). For the sake of time management, have one member of your team do the 2-20 pipet series and the other member of the team work with the 20-200 ul pipet.

Depending on the size of the paper and number of runs, you may have more than one piece of paper. Use a pencil to place a series of ticks 1 cm apart, 1 cm from the edge. 10 ticks should be in one row and to the right of the row, ID what the ticks will be see below volumes (note diagram on board for clarification) . About 2 cm below the first series of ticks, repeat the procedure for the next volume in your pipet series.

Using a 2-20 ul pipette place ten drops of 2 ul from left to right. Space them out evenly lining up near ticks. At the far right of the series, label it : 2 ul, 2-20 pipette.

On the next line, repeat the above, but set the pipette for 5 ul.
Repeat again for 10 ul and 20 ul amounts and **LABEL EACH ROW ACCORDINGLY.**

For the 20-200 pipette, try the process with 20, 50, 100 and 200 ul.
Be sure to use another piece of paper for this procedure and to label each row properly and DO NOT forget your name!!!.

Please note, as the volumes get larger, you may need to space the dots out further apart. Also be careful to not disturb the dots in ANY way while they are drying. This could distort the dot shape and the subsequent evaluation.

While we could use this process for 100-1000 ul pipettes, the amount of dye, soaking through the paper, etc. can all affect the quality of the dot. Fortunately, as the volume gets larger, there are other ways to assess the accuracy of the transfer technique (see later labs for details on this topic).

Once you have complete the pipette exercises, post your filter sheets on the wall as directed. Be sure to put your sheet next to other comparable sheets (similar pipet volumes) and remember to put you name on the sheet.

So….what about accuracy and precision. Well, the definitions are as follows:

Accuracy: Indication of how close a measurement is to the actual value.

Using volumetric devices to assess volumetric devices can reveal this (2,3,5 exercise idea). Another technique involves using water and its unique relationship in the metric system. Recall that 1 ml of water weighs 1 gram, which takes up 1 cubic centimeter of space. This is assessed by comparing the pipetting amounts to the actual amount that should have been transferred. In a upcoming lab, we will assess this by comparing the mass of water transferred to the volume of water transferred and figuring out the accuracy of the volume transferred using micropipettes. The previous procedure with serological pipettes is an example of this idea FYI.

Precision: How reproducible is the measurement?

This is a reflection of technique (for the most part). Sometimes the device is damaged, but for most pipettes, the damage in micropipettes is such that they may be very precise but not accurate. What usually happens is a device gets damaged and does not deliver the correct amount, but this damage is not random. The device usually always under or over delivers the exact same amount. So, if off by 0.1 ml, it will very precisely always give you 0.1 ul too much, every measurement, every time. In other words, it will very precisely give you the same volume, but it will not be accurate.

Assuming your technique is good, the dots should all be the same size (with in a given "volume series). If this is this case, your technique has good precision. Also, your dots should be the same size as compared to other folks. If this is the case, it is likely, your device is very accurate. If not, it is possible there is some issue with your pipette. Notify the instructor if tis appears to be the case and in later labs, we will revisit the idea of accuracy and precision as needed.

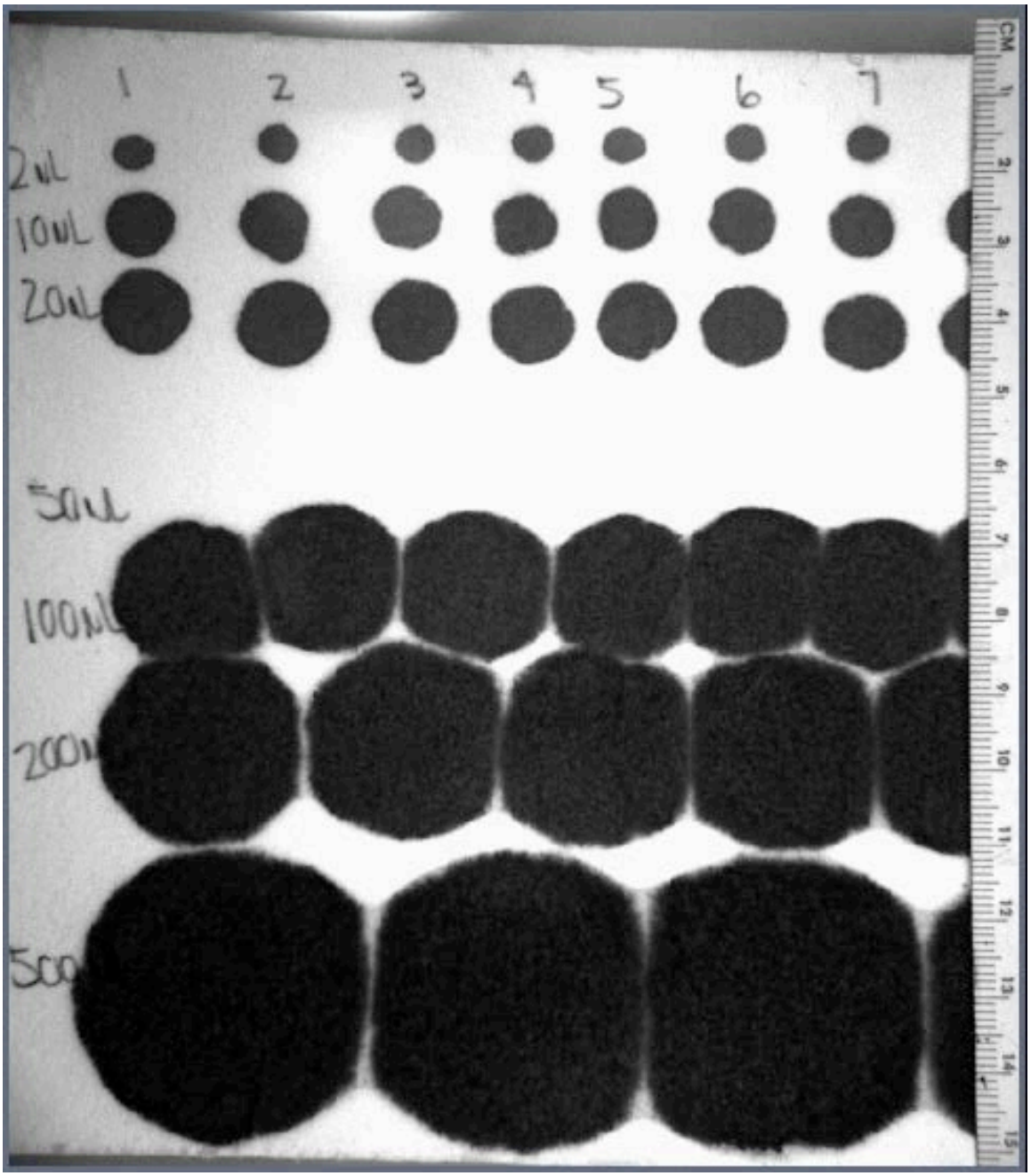

Image of loading dye added to filter paper. Note size of dots and try to add your samples far enough apart to avoid overlap (note scale bar/ ruler in cm). The 50 ul sample were not added to this sample FYI.

NOTE….SAVE ALL OF THE SEROLOGICAL PIPETTES USED IN THIS PROCEDURE. THEY CAN BE RE-USED FOR PROTOCOLS WHERE STERILITY IN NOT AN ISSUE.

For disposal of serological pipettes, a special tray is used. This tray often has sterilizing solution in it. Plastic pipets are usually autoclaved and discarded and glass ones are often reused. Regardless of fate, the pipets should NEVER be placed directly into autoclave or other bags for their disposal. Their long shape and pointy tips (especially if broken) can easily pierce the bag, resulting in possible injury.

Key to cover picture. Remember to look at on-line picture as this may make seeing the equipment easier.

1. Micropipette (one of three) in a free standing Plexi-glass rack.
2. Serological pipet pump. Electronic device that allows for aspirating and expelling of samples by pressing buttons. Good for reducing fatigue, repetitive stress injuries.
3. Sterile (wrapped) serological pipettes. Different volumes.
4. Box of pipette tips. Note autoclave tape suggesting they were once sterile.
5. Multi-channel pipet. Note the large, "off white" base partially obscured by the other items.
6. Micropipette, full view.
7. Beaker with a few transfer pipettes. Not wrapped, so not sterile. Come in a HUGE variety of shapes, volumes, etc.
8. Electronic micropipette. Likely has a rechargeable battery and a digital display.
9. Multi-channel pipette on computer screen. Not an especially safe place to keep the device!