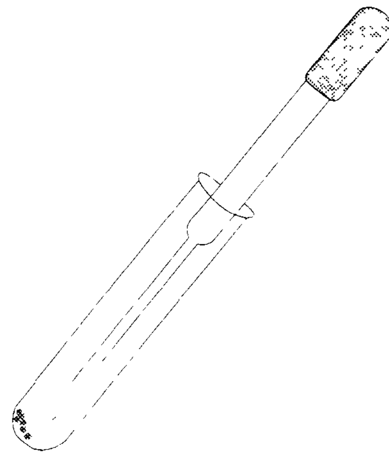
General Terms and Fundamental Techniques

The Pasteur pipets we use are disposable, which is to say that when they become too difficult to clean or broken toss them and get another. As long as you are not profligate there will always be some for you. To be useful the Pasteur pipet should be fit snuggly with the 'red' or the 'latex' rubber-dubber, the 'blue' has too much volume, the 'black' is hopelessly small. Tight fit of the rubber-dubber is critical, it should hold the solution so that it does not drop out until you squeeze.



The main use of the pipet is to decant the supernatant off the precipitate (ppt). Though 'decant' really means to ***pour*** off (which, by the way, is often possible), when the ppt is not packed well enough by centrifugation the supernatant can still easily be sucked away with the pipet. After centrifugation you will note that the ppt pellet will be packed to one side at the bottom of the tube. ***First exhaust the pipet*** by squeezing the rubber-dubber then place it on or near the bottom of the tube opposite the pellet with tube tilted as shown Then slowly relax the squeeze. Usually this picks up the solution/supernatant/decantate leaving the ppt. Practice this patiently a few times: you'll be a pro by Friday

Centrifuging (for fun and profit). ***Always*** balance the tube containing your sample to be centrifuged by another of the same size and shape and thickness containing very nearly the same amount of water as you have sample solution—placed ***opposite*** your sample in the centrifuge. This will avoid the raucous and dangerous vibrations which accompany disbalance. If the centrifuge vibrates when run empty ask your instructor to fix it. Since different sized rubber spacer plugs (not visible) are placed in the metal holder-tubes so that different sizes of test tubes may be used, the metal holder tubes should have been matched in weight with their opposite, but not necessarily with their adjacent tubes. Please don't rearrange these unadvisedly. So your tubes, sample and counterweight, should fit into the metal holder tubes to equal extent. Never let your glass tubes sit against each other as they will surely break and fling glass during centrifugation

How long one should centrifuge depends on the sample Thirty seconds at speed will pack most, but some may require two or more minutes. Clarity (no mention here of color) of the supernatant solution is the best measure of completion. Sometimes total clarity cannot be reached but don't give up without some extended centrifugation. As mentioned above, most ppts pack so well that you could pour the supernatant/decantate off. In other cases, as you will learn, any kind of agitation breaks the pellet up.

To "wash" a ppt means ***three*** operations:

1. add to the ppt the wash solvent which may be water or a particular solution. Always stir to resuspend the ppt but heat only if specified;
2. centrifuge;
3. decant the supernatant, which may be combined with some previous decantate or discarded. This leaves you the washed ppt.

To "check" for complete precipitation you will need to clarify the supernatant by centrifugation but you need not decant. Then add a drop of the precipitating reagent gently and observe where it mixes with the solution to look for cloudiness—which would indicate more reagent is needed. If no cloudiness occurs precipitation is complete.

Filling your kit. You will have your own personal reagent kit for the semester. You should be sure all 10 bottles are clean and legibly labeled for the 10 'kit' reagents. The old labels are left on from semester to semester to minimize intermixing of these reagents. If you are picky you can relabel but use the same bottle for the same reagent. Bottles previously used for 8M NaOH and TA get very grubby, just be sure not to use them with any other reagents. Others may be switched if carefully washed. When cleaning, disassemble the dropper cap and wash thoroughly with soap, tap water and finally DI water. Be sure that the reassembled dropper cap will hold water, otherwise you may need to seek to replace the rubber. When filling these bottles resist the urge to fill them full. All these solutions degrade the rubber, some quickly, and too-full bottles are soon messed up. Also never use the dropper on any reagent bottle, nor your Pasteur pipet so as to get the solution onto the rubber; keep it in the glass.

The "unknown tube": You will turn in a tube one at a time for your instructor to put your unknowns in. The best policy is to place your tube for the next unknown when you pick up an unknown. Each instructor has his own specifications for these as to size, where to label, what to label and where to place it. You must meet his/her specifications. Your instructor will simply add the "unknown" to the tube without any cleaning, so if you want a clean sample give a clean tube.