Austin Community College
Biotechnology Program
Basic Equipment Standard Operating Procedures
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SOP AUT-001
OPERATION OF VWR/THERMO SCIENTIFIC STERILEMAX AUTOCLAVE
Revision D, October 9, 2019

Title: Operation of VWR/Thermo Scientific Sterilemax Autoclave

1.0 SCOPE AND APPLICATION:

This SOP outlines the policy and procedure for operation of the Sterilemax autoclave.

2.0 SUMMARY OF METHOD

MATERIALS	COMMENTS
1. Sterilemax autoclave 2. 4-7 L deionized water 3. Autoclave indicator tape 4. Cleaning solution and scouring pad	
PART A: OPERATION	
1. Plug autoclave into a 115-V outlet. Turn on autoclave using switch on bottom right edge of front of machine.	
2. Check water level by opening reservoir on top right side. Water should be at least six inches from bottom of reservoir and should not be discolored or cloudy.	This autoclave automatically cycles water back into the reservoir, so the water is often contaminated. Water should be drained and replaced at least once a week.
3. If water appears contaminated, empty the reservoir by following instructions in Part C. If water level is too low, add deionized water until water is at least 6 inches high. Replace lid on reservoir.	
4. Open chamber door by pushing the handle to the left until it is out of the way of the door. Squeeze the door release panel on bottom left edge of door to release the door fully. Pull forward to open.	
5. The autoclave chamber always contains a base rack that covers the bottom of the chamber and has two sets of rails for shelves along the sides. Never operate the autoclave without the base rack. You may also use up to three trays, one that goes on the bottom, and two that fit on the rails.	
6. Select items to be autoclaved: a. Make sure all materials are autoclave-safe: many plastics may melt, and some chemicals are not heat-stable. b. Make sure all items will actually fit	

inside the autoclave chamber. Test glassware for fit before making a solution in the glassware. c. Open containers and loose items such as bottle caps or forceps may be covered loosely in foil. d. Bottles containing liquid should be filled no more than halfway to prevent boiling over. e. Caps on bottles should always be left loosened to prevent bottles from exploding. f. When autoclaving media, it is a good idea to place the containers inside a bucket to catch any liquid that may boil over. This protects the autoclave from a mess and possible damage. g. Always put a small piece of autoclave indicator tape on each item before autoclaving. Stripes or words will appear on the tape when sterilizing conditions have been met. 7. Load items to be autoclaved into chamber. Do not pack items too tightly together, or steam will not be able to move freely between them. 8. Close the chamber door and swing the handle to the right until its axis is snugly placed in the door. Turn the handle clockwise until it is difficult to turn further. Do not over-tighten, or it will be hard to open. 9. Press “start” to choose a program. There are four programs to choose from. a. If you are sterilizing liquids, choose the **liquid** cycle. This will heat to a higher temperature than the other cycles and exhaust more slowly to prevent evaporation and splashing. **Be certain that all caps have been loosened or containers can explode under high pressure!** b. For wrapped materials (such as sterilizing pouches full of instruments or for biohazard-contaminated materials in a biohazard bag), choose the **wrapped** cycle. This will exhaust quickly. c. For unwrapped materials (such as empty glassware), choose the	This autoclave will not fit flasks larger than two liters in size, or Wheaton bottles larger than one liter.

unwrapped cycle. This will exhaust very quickly. d. The **packs** cycle is designed for lab packs, which are containers full of biohazardous sharps and Plaster of Paris. You will not need to use this cycle in our lab. 10. Once you have pressed the button corresponding to the program you want, the parameters of the program will appear. You can change these if you wish, but in most cases you will want to leave the default parameters. 11. Press “start” and the program will begin. 12. When the sterilization is complete, the machine will beep and display an instruction. a. For the wrapped and unwrapped cycles, you will be instructed to open the chamber door to allow the chamber to exhaust. This is to allow the items inside to dry off. b. For the liquid cycle, the exhaust has already occurred. The instruction will be to press “start” to clear the program so the autoclave is ready to use again. 13. CAUTION! When opening the chamber door after an autoclave cycle, steam may escape and surfaces just inside the door may be very hot! Stay to the right side of the autoclave and keep your hand and arm away from the opening so you are not burned by steam escaping. 14. Open chamber door by rotating the handle counter-clockwise to loosen, and then moving it to the left and out of the way of the door. 15. Squeeze the door release panel on bottom left edge of door to release the door fully. Carefully pull forward to open, being sure to avoid any escaping steam or hot surfaces. 16. Leave the door slightly ajar and allow the drying cycle to complete for “dry” items. Avoid removing hot items right away; allow them to cool down for 10-15 minutes before attempting to remove anything.	When autoclaving “dry” items such as empty glassware, you do need to open the door as soon as possible so the exhaust helps to dry off the materials. When autoclaving liquids, it is not as important to open the door just after the program, since the exhaust happens automatically without the door being opened.

17. Remove trays or items using insulated gloves or hot hands to avoid burns. Place on a lab bench or cart to cool to a reasonable temperature before attempting to use.

PART B: OPERATION CHECKLIST

- ☐ Check water in reservoir
- ☐ Check that base rack is in place
- ☐ Loosen caps on bottles and DO NOT OVERFILL (more than 1/2 full)
- ☐ Wrap loose or open items in foil
- ☐ Mark items with indicator tape
- ☐ Load chamber, close door, and start cycle
- ☐ Open door for drying cycle (if required)
- ☐ Allow items to cool with door open for 10-15 minutes
- ☐ Remove your items when finished

PART C: MAINTENANCE

1. At least once weekly, the reservoir should be drained completely of water and refilled. To drain, open the chamber door when cool. Locate the drain hose and find the working end (has a white plastic gasket).
2. Place the non-working end into a bucket. Insert the working end of the drain hose into the drain plug, located inside the door frame on the lower right.
3. Reservoir will begin draining immediately. Drain completely and remove hose by pressing on release button (just below the drain plug).
4. Refill reservoir with 4-7 liters of deionized water.
5. At least once monthly, the autoclave chamber should be scrubbed using a plastic scouring pad and a special chamber cleaner. The cleaner residue should be wiped out with a damp towel until it is no longer soapy. The chamber can also be

rinsed with deionized water and drained using the drain hose to completely remove the cleaner residue.	

SOP AUT-003
USE AND MAINTENANCE OF STERILMATIC AUTOCLAVE
Revision B, October 9, 2019

Title: Use and Maintenance of Sterilmatic Autoclave

1.0 SCOPE AND APPLICATION:

This SOP outlines the policy and procedure for using the Sterilmatic autoclave.

2.0 SUMMARY OF METHOD

MATERIALS	**COMMENTS**
1. Sterilmatic autoclave 2. 4 L deionized water 3. Autoclave indicator tape	This autoclave is hard wired to electricity, so it doesn't have to be plugged in or turned on manually.
PART A: OPERATION	The chamber door should be left open between cycles.
4. Make sure chamber drain dial is in closed position (parallel to the floor). Pour 4 L of deionized water into the chamber. If needed, add more water until it reaches the fill line.	If you get a low water warning during the autoclave cycle, you may not have filled the chamber with enough water! Open the chamber when it is safe to do so, and fill with more water until it is at the fill line.
5. Place the bottom baffle into the bottom of the chamber. This baffle serves as a base shelf. You may put other racks or trays on top of it. There are rails on the sides of the chamber that will fit two more racks.	
6. Select items to be autoclaved: a. Make sure all materials are autoclave-safe: many plastics may melt, and some chemicals are not heat-stable. b. Make sure all items will actually fit inside the autoclave chamber. Test glassware for fit before making a solution in the glassware. c. Open containers and loose items such as bottle caps or forceps may be covered loosely in foil. d. Bottles containing liquid should be filled no more than halfway to prevent boiling over. e. Caps on bottles should always be left loosened to prevent bottles from exploding. f. When autoclaving media, it is a good idea to place the containers inside a bucket to catch any liquid that may boil over. This protects the autoclave from a mess and	This autoclave will fit larger and taller glassware, such as 2 liter Wheaton bottles and 4 liter Erlenmeyers.

possible damage. g. Always put a small piece of autoclave indicator tape on each item before autoclaving. Stripes or words will appear on the tape when sterilizing conditions have been met. 7. Load items to be autoclaved into chamber. Do not pack items too tightly together, or steam will not be able to move freely between them. 8. Grasp the door handle and hold it in a vertical position while pulling down until bottom of door rests in the door opening. Make sure the rubber seal is flush with the door. Then push door handle down to engage the lock. 9. Select the correct exhaust type. “Liquids” is to be used for any liquids, while “Instruments” can be used for all other items. 10. Set the temperature to 121ºC. 11. Turn the timer to start operation. Timer should always be set to at least 15 minutes, as this is the minimum time for sterilization to be effective. 12. When the chamber reaches the selected temperature and pressure, the timer will begin and a red light on the top right of the front panel will light up. 13. When cycle is complete, allow the chamber pressure gauge to drop to zero before attempting to open the door. CAUTION! When opening the chamber door after an autoclave cycle, steam may escape and surfaces just inside the door may be very hot! Stand to one side of the autoclave and keep your hand and arm away from the opening as much as possible, so you are not burned by steam escaping. 14. Open door by pulling up on the door handle. Stay to the side to avoid steam burns. 15. Leave the door slightly ajar and allow the drying cycle to complete for “dry” items. Avoid removing hot items right away; allow them to cool down for 10-15 minutes	“Liquids” will result in a slow exhaust that will protect your liquids from boiling over. “Instruments” results in a fast exhaust that will allow your items to dry quickly. This autoclave doesn’t give any indicator sounds to tell you when the cycle is done. You have to visually check the timer and pressure gauge.

before attempting to remove anything. 16. Remove trays or items using insulated gloves or hot hands to avoid burns. Place on a lab bench or cart to cool to a reasonable temperature before attempting to use. 17. Turn the drain dial perpendicular to the floor to drain the chamber, and leave it in the open position. Leave the chamber door open when finished. **PART B: CHECKLIST** ☐ Close drain valve and add deionized water to the chamber to the fill line (approximately 4 L) ☐ Place bottom baffle into chamber ☐ Loosen caps on bottles and DO NOT OVERFILL (more than 1/2 full) ☐ Wrap loose or open items in foil ☐ Mark items with indicator tape ☐ Load chamber and close door ☐ Choose appropriate cycle (slow or fast) ☐ Start timer ☐ When pressure is at 0 atm, it is safe to open door ☐ Open door for drying cycle (if required) ☐ Allow items to cool with door open for 10-15 minutes ☐ Remove your items when finished ☐ Open the drain valve to drain the chamber **PART C: MAINTENANCE** At the end of each day, the chamber should be drained completely. Turn the drain dial perpendicular to the floor and the chamber will drain. Leave the drain dial in the open position and the chamber door open.	This autoclave is connected directly to a drain pipe which will remove the chamber water when drained.

SOP BAL-001
OPERATION AND CALIBRATION OF ACCULAB MODEL VI-200 ELECTRONIC BALANCE
Revision A, February 22, 2011

Title: Operation and calibration of Acculab V1-200 Balance

1.0 SCOPE AND APPLICATION:

This SOP outlines the policy and procedure for the proper operation and calibration of the **Acculab Model VI-200 Balance**.

2.0 SUMMARY OF METHOD

MATERIALS	**COMMENTS**
1. VI-200 balance 2. standard 200 g mass	
PROCEDURE **PART A: CALIBRATION**	
1. Turn on balance and allow it to warm up for at least 30 minutes.	Use only a 12V AC adapter with a negative tip to supply power. Unplug when not in use.
2. Remove all items from the weighing pan and tare the balance by pressing the **TARE** key.	
3. Check that the balance is in gram mode, indicated by a stable arrow on the display next to the **g** symbol on the casing.	If the balance is not in gram mode, press and release the **CAL/MODE** key until the arrow is next to **g**.
4. After observing a stable zero reading (0.00) press and hold the **CAL/MODE** key. After four seconds the unit will beep and the calibration weight (+200) will appear on the display. Release the **CAL/MODE** key.	
5. Gently place the 200 gram standard mass on the weighing pan.	Do not disturb the balance during calibration, and avoid vibrations and air currents.
6. The + sign will disappear from the display. Wait seven to ten seconds and the unit will beep.	If the calibration weight value remains on the display, an improper weight has been used. Select the proper weight (200g) and repeat the calibration.
7. The displayed weight (200) will disappear and then reappear as an active value. Calibration is now complete.	
PART B: OPERATION	
1. Remove all items from the weighing pan and tare the balance by pressing the **TARE** key.	Reagents can be weighed directly into any vessel as long as it does not weigh more than

2. Check that the balance is in gram mode, indicated by a stable arrow on the display next to the **g** symbol on the casing. 3. Place a weigh boat, weigh paper, or small vessel (such as an Erlenmeyer flask) on the balance pan. 4. Press the **TARE** key to tare the weight of the weighing vessel. 5. Place the substance to be weighed into the weighing vessel and allow the value to stabilize on the display. 6. Record the mass of the substance, remove the weighing vessel and contents from the balance and turn off. 7. Check that the entire balance and the area around it are clean and dry.	200 grams (the maximum capacity of this model). If weighing a chemical, use a clean, dry spatula to transfer it into the vessel, or if a free-flowing solid, gently sift it from the container. Record any error messages or drifting mass values in the comments section of the log. If the balance was dirty or wet or the balance area was messy when you began working, record that in the comments section.

SOP BAL-002
OPERATION AND CALIBRATION OF
METTLER-TOLEDO EXCELLENCE XS MODEL ANALYTICAL BALANCE
Revision A, May 14, 2010

Title: Operation and calibration of M-T Excellence analytical balance

1.0 SCOPE AND APPLICATION:

This SOP outlines the policy and procedure for the proper operation, calibration and maintenance of the M-T Excellence analytical balance.

2.0 SUMMARY OF METHOD

MATERIALS	**COMMENTS**
1. VI-200 balance 2. standard 200 g mass	Use of this SOP indicates that the user has read the M-T Excellence XS user manual. The manual must be read prior to use of this SOP.
PROCEDURE **PART A: CALIBRATION** The Excellence XS analytical balance has fully automatic calibration functions, so it needs to be calibrated by hand very rarely.	
PART B: OPERATION	
1. **CAUTION**: Do not overload balance. Maximum capacity is 220 g.	Do not use the **ZERO** key for taring. This is only used for initial setup of the balance. Do not disturb the balance during operation, and avoid vibrations and air currents.
2. Place a weigh boat or paper on the weighing pan, close all draft shields, and tare the balance by pressing the **TARE** key. “Net” will appear in the display to indicate that all displayed weights will be net values.	If weighing a chemical, use a clean, dry spatula to transfer it into the vessel.
3. Open the draft shield and insert the items to be weighed. Never sift chemicals from a container directly into the analytical balance. Spillage could devastate the instrument. Instead, carefully transfer material using a spatula.	Record any error messages or drifting mass values in the comments section of the balance log, found in the equipment log book. If the balance was dirty or wet or the balance area was messy when you began working, record that in the comments section.
4. Close the draft shield and wait for the **stability detector icon**, a small circle, disappears from the display. The presence of this icon indicates that the balance is NOT stable and you should wait for it to disappear.	
5. Record the mass and remove all items from the weighing pan. Turn off balance by pressing the **ON/OFF** key and holding down until balance is off.	

6. Check that the entire balance and the area around it are clean and dry. Remove any material from the inside of the balance by gently sweeping it out with a brush. Avoid pressing down on the balance pan while cleaning. Do not use water to clean. Wipe any material away from the area around the balance. Clean spatulas or other weighing tools thoroughly with soapy water and dry completely before replacing.	Never replace a used spatula to its storage container before cleaning it thoroughly.

SOP BAL-003
OPERATION AND CALIBRATION OF
OHAUS AP110 ANALYTICAL BALANCE
Revision A, September 11, 2007

Title: Operation and calibration of Ohaus AP110 analytical balance

1.0 SCOPE AND APPLICATION:

This SOP outlines the policy and procedure for the proper operation, calibration and maintenance of the Ohaus analytical balance.

2.0 SUMMARY OF METHOD

MATERIALS	**COMMENTS**
1. Ohaus AP110 balance 2. standard 100 g mass **PROCEDURE** **PART A: CALIBRATION** 1. Make sure there is no load on the balance pan and close the draft shield. 2. Hold down the **ON/TARE** button until "MENU" appears. 3. Press the **ON/TARE** button once and "AUTO" will appear. 4. Press the **MODE** button twice until "User" appears. 5. Press the **ON/TARE** button and "100.0000" should appear. This is the value used to last calibrate the balance. Press **ON/TARE** repeatedly to confirm each digit of this number is correct, then press again and "0 g" should appear. 6. Press **ON/TARE** once more to start zero mass calibration. "-C-" should appear and the balance will initiate calibration. 7. When "CAL 100g" appears, place a 100-g mass on the balance pan. Press **ON/TARE** to initiate 100 g calibration. 8. "200.0000" should appear. Calibration is complete. **PART B: OPERATION** 1. Turn balance on by pressing the **ON/TARE** button.	Use of this SOP indicates that the user has read the Ohaus AP110 user manual. The manual must be read prior to use of this SOP. **CAUTION**: Do not overload balance. Maximum capacity is 100 g.

2. Place a weigh boat or paper on the weighing pan, close all draft shields, and tare the balance by pressing the **TARE** key.	
3. Open the draft shield and insert the items to be weighed. Never sift chemicals from a container directly into the analytical balance. Spillage could devastate the instrument. Instead, carefully transfer material using a spatula.	If weighing a chemical, use a clean, dry spatula to transfer it into the vessel. Record any error messages or drifting mass values in the comments section of the balance log, found in the equipment log book. If the balance was dirty or wet or the balance area was messy when you began working, record that in the comments section.
4. Close the draft shield and wait for the **stability indicator**, a small “s”, to appear on the left of the display.	
5. Record the mass and remove all items from the weighing pan.	
6. Turn off balance by pressing the **ON/OFF** key and holding down until balance is off.	
7. Check that the entire balance and the area around it are clean and dry. Remove any material from the inside of the balance by gently sweeping it out with a brush. Wipe any material away from the area around the balance.	Avoid pressing down on the balance pan while cleaning. Do not use water to clean.
8. Clean spatulas or other weighing tools thoroughly with soapy water and dry completely before replacing.	Never replace a used spatula to its storage container before cleaning it thoroughly.

SOP BAL-004
OPERATION AND CALIBRATION OF METTLER-TOLEDO PL1502-S ELECTRONIC BALANCE
Revision A, February 22, 2011

Title: Operation and calibration of Mettler-Toledo PL1502-S Balance

1.0 SCOPE AND APPLICATION:

This SOP outlines the policy and procedure for the proper operation and calibration of the **Mettler-Toledo PL1502-S Balance**.

2.0 SUMMARY OF METHOD

MATERIALS	**COMMENTS**
1. PL1502-S balance 2. standard 200 g mass	
PROCEDURE **PART A: CALIBRATION**	
1. Turn on balance and allow it to warm up for at least 30 minutes.	Do not disturb the balance during calibration, and avoid vibrations and air currents.
2. Remove all items from the weighing pan and tare the balance by pressing the **TARE** key.	
3. Press the CAL key to start calibration.	
4. The maximum weight value, 200.000 grams, will flash on the LCD.	
5. Gently place the 200 gram standard mass on the weighing pan.	
6. When 0.00 g flashes on the LCD, remove the 200 g weight.	
7. The adjusting is finished when the message CAL done appears on the display, followed by 0.00 g.	
PART B: OPERATION	
1. Place a weigh boat, weigh paper, or small vessel (such as an Erlenmeyer flask) on the balance pan.	Reagents can be weighed directly into any vessel as long as it does not weigh more than 200 grams (the maximum capacity of this model).
2. Press the **0/T** key to tare the weight of the weighing vessel.	If weighing a chemical, use a clean, dry spatula to transfer it into the vessel, or if a free-flowing solid, gently sift it from the container.
3. Place the substance to be weighed into the weighing vessel and allow the value to	

stabilize on the display. The stability detector “º” will disappear when the reading is stable. 4. Record the mass of the substance, remove the weighing vessel and contents from the balance and turn off. 5. Check that the entire balance and the area around it are clean and dry.	

SOP BAL-005
OPERATION AND CALIBRATION OF OHAUS SCOUT PRO ELECTRONIC BALANCE
Revision C, June 6, 2012

Title: Operation and calibration of Ohaus Scout Pro SP402 Balance

1.0 SCOPE AND APPLICATION:

This SOP outlines the policy and procedure for the proper operation and calibration of the **Ohaus Scout Pro Balance**.

2.0 SUMMARY OF METHOD

MATERIALS	**COMMENTS**
1. Scout Pro balance 2. standard 200 g mass	
PROCEDURE **PART A: CALIBRATION**	
1. Remove all items from the weighing pan.	Do not disturb the balance during calibration, and avoid vibrations and air currents.
2. Press and hold the **ON/ZERO** key until "MENU" appears on the display.	
3. After you release the **ON/ZERO** key, "CAL" will display on the screen.	
4. Press the **ON/ZERO** key again to start calibration.	
5. "C" will appear on the display and the balance will calibrate to mass = 0 g. Do not place anything on the balance at this time.	The 0 gram calibration step happens automatically and very quickly, so it may appear that the balance is skipping over it.
6. Press the **ON/ZERO** key to display "C 200" (flashing).	
7. Gently place the 200 gram standard mass on the weighing pan.	
8. Press the **ON/ZERO** key to calibrate with the 200 gram mass. "C" will flash on the screen for a moment, then the screen will display "done" briefly before going into live measurement mode.	
9. Remove the 200 gram mass.	
PART B: OPERATION	
1. With the balance on, place a weigh boat, weigh paper, or small vessel (such as an Erlenmeyer flask) on the balance pan.	

2. Press the **ON/ZERO** key to tare the weight of the weighing vessel. 3. Place the substance to be weighed into the weighing vessel and allow the value to stabilize on the display. The stability detector "*" will disappear when the reading is stable. 4. Record the mass of the substance, remove the weighing vessel and contents from the balance and turn off by pressing and holding the **ON/ZERO** key. 5. Check that the entire balance and the area around it are clean and dry.	Reagents can be weighed directly into any vessel as long as it does not weigh more than 200 grams (the maximum capacity of this model). If weighing a chemical, use a clean, dry spatula to transfer it into the vessel, or if a free-flowing solid, gently sift it from the container.

SOP CEN-001
USE OF EPPENDORF 5417R MICROCENTRIFUGE
Revision A, April 22, 2011

Title: Use and Maintenance of Eppendorf 5417R Microcentrifuge

1.0 SCOPE AND APPLICATION:
This SOP outlines the use and maintenance of the Eppendorf 5417R refrigerated microcentrifuge.

2.0 SUMMARY OF METHOD

PROCEDURE	COMMENTS
1. Turn centrifuge on with main power switch.	
2. Press the LID button to open the centrifuge lid.	
3. Open the rotor cover by rotating the center knob and pulling up on the cover.	
4. Load tubes into centrifuge in a balanced configuration.	This will reduce the risk that the centrifuge becomes unbalanced and vibrating. Additionally, samples may be thrown out if unbalanced, causing extreme damage.
5. Replace rotor cover. Press down on center knob and rotate to lock in place.	
6. Close centrifuge lid and press down to lock in place.	
7. Set temperature, time, and speed values to the appropriate settings using the cursor keys. To change speed units from “rpm” to “rcf”, press both cursor keys simultaneously.	
8. Press the START key to begin the run.	
9. If needed, press the STOP key to stop the run before the elapsed time. Allow the rotor to slowly decrease speed.	
10. When the centrifuge can be safely opened, the OPEN key green LED will light up.	**Do not open the lid until rotor stops.**
11. Press OPEN to open the lid and remove tubes.	

SOP CEN-002
USE OF EPPENDORF 5804R CENTRIFUGE
Revision A, May 31, 2010

Title: Use and Maintenance of Eppendorf 5804R Tabletop Centrifuge

1.0 SCOPE AND APPLICATION:
This SOP outlines the use and maintenance of the Eppendorf 5804R refrigerated centrifuge.

2.0 SUMMARY OF METHOD

PROCEDURE	COMMENTS
1. Turn centrifuge on with main power switch (on right side of unit).	
2. Press the power button (if off, it will be lit red; if on, it is lit green).	
3. Press the LID button to open the centrifuge lid, if it is not already open.	
4. Determine the correct rotor for your application. If it is installed, proceed to next step. If not installed, see Appendix for instructions on switching out the rotor.	
5. Open the cover of the rotor by lifting off or rotating and lifting, depending on the design of the rotor.	
6. Set temperature, time, and speed values to the appropriate settings using the cursor keys. To change speed units from “rpm” to “rcf”, press the speed button to change the symbol displayed.	
7. You may need to wait for the centrifuge to cool to a chilled temperature before starting. If so, close the lid and then allow the centrifuge to cool down before loading.	
8. Load tubes or plates into centrifuge rotor in a balanced configuration. 9. Replace rotor cover. 10. Close centrifuge lid and press down to lock in place; you will hear a click. 11. Press the START key to begin the run. 12. If needed, press the STOP key to stop the run before the elapsed time. Allow the rotor to slowly decrease speed.	Balance tubes and plates weighing an equal amount on opposite sides of the rotor. This will reduce the risk that the centrifuge becomes unbalanced and vibrating. Additionally, samples may be thrown out if unbalanced, possibly causing damage to the centrifuge and loss of samples.

13. Press LID to open the lid and remove tubes or plates. 14. Replace the rotor cover and turn off centrifuge when finished. 15. It is best to leave the centrifuge lid open when not in use, to prevent microbial growth inside the chamber. **APPENDIX: Changing rotors** 1. Remove the lid of the rotor. 2. Insert rotor key (simply a hex/Allen wrench with handle) into the top of the rotor and rotate to unfasten. 3. Lift the rotor out of the centrifuge. The rotor may stick; wiggle the rotor while firmly lifting to unstuck. 4. Place the new rotor in the centrifuge, aligning the center with the post in the middle of the centrifuge. 5. Use the rotor key to fasten the rotor down. 6. Store the extra rotor safely, preferably inside a padded box.	**Do not open the lid until rotor stops.**

SOP CEN-003
VWR CLINICAL 200 CENTRIFUGE
Revision A, May 31, 2011

Title: Use and Maintenance of VWR Clinical 200 Centrifuge

1.0 SCOPE AND APPLICATION:
This SOP outlines the use and maintenance of the VWR Clinical 200 centrifuge.

2.0 SUMMARY OF METHOD

PROCEDURE	**COMMENTS**
1. Turn centrifuge on with main power switch (on left underside of unit).	
2. Press the OPEN button to open the centrifuge lid, if it is not already open.	
3. Load 15-mL tubes into centrifuge rotor in a balanced configuration.	Balance tubes weighing an equal amount on opposite sides of the rotor. This will reduce the risk that the centrifuge becomes unbalanced and vibrating. Additionally, samples may be thrown out if unbalanced, possibly causing damage to the centrifuge and loss of samples.
4. Close centrifuge lid and press down to lock in place; you will hear a click.	
5. Set the speed by pressing the rpm/rcf key and using the up and down arrows to set the speed. To switch between the units of speed, press the rpm/rcf key twice.	
6. Set the time by pressing the time key and using the up and down arrows.	
7. Press the START key to begin the run.	
8. If needed, press the STOP key to stop the run before the elapsed time. Allow the rotor to slowly decrease speed.	**DO NOT OPEN THE LID UNTIL ROTOR STOPS.**
9. Press LID to open the lid and remove tubes.	
10. Turn off centrifuge when finished.	
11. It is best to leave the centrifuge lid open when not in use, to prevent microbial growth inside the chamber.	

SOP CEN-004
USE OF VWR GALAXY 16D MICROCENTRIFUGE

Title: USE AND MAINTENANCE OF VWR GALAXY 16D MICROCENTRIFUGE

1.0 SCOPE AND APPLICATION:
This SOP outlines the use and maintenance of the VWR Galaxy 16D microcentrifuge.

2.0 SUMMARY OF METHOD

PROCEDURE	**COMMENTS**
1. Turn centrifuge on with main power switch (on back of unit).	
2. Lift lid to open.	
3. Load 1.5-2.0 mL tubes into centrifuge rotor in a balanced configuration. Replace rotor cover.	Balance tubes weighing an equal amount on opposite sides of the rotor. This will reduce the risk that the centrifuge becomes unbalanced and vibrating. Additionally, samples may be thrown out if unbalanced, possibly causing damage to the centrifuge and loss of samples.
4. Close centrifuge lid.	
5. Set the speed using the up and down arrows next to the speed display, up to 14,000 rpm. Toggle between rpm and g-force using the mode key.	
6. Set the time using the up and down arrows next to the time display (in minutes).	
7. Press the START key to begin the run. Lid will lock during the run and will not open until the rotor stops.	**DO NOT OPEN THE LID UNTIL ROTOR STOPS.**
8. If needed, press the STOP key to stop the run before the elapsed time. Allow the rotor to slowly decrease speed.	
9. The lid will unlock and the unit will beep when the run is finished.	
10. Turn off centrifuge when finished.	

SOP DTR-001
DIGITAL TEMPERATURE RECORDER
Revision A, June 8, 2011

Title: Use and Maintenance of Digital Temperature Recorder

1.0 SCOPE AND APPLICATION:

This SOP outlines the policy and procedure for use of a digital temperature recorder.

2.0 SUMMARY OF METHOD

MATERIALS 1. Digital temperature recorder 2. Probe **PART A: OPERATION** 1. Insert the temperature probe into the recorder. 2. Press the round yellow power button on the recorder to operate. Timer will start when unit is turned on. 3. Situate probe in the area in which you want to measure temperature. Close the door to freezers, incubators, etc, on the cord gently so you do not damage the cord. 4. Allow the probe to equilibrate to the temperature. This may take a few minutes. 5. Record temperature for the desired time period. 6. Remove probe and turn off recorder.	

SOP ELC-002
MINI-PROTEAN CELL
Revision A, February 22, 2011

Title: Operation of Bio-Rad Mini-Protean Vertical Electrophoresis System

1.0 SCOPE AND APPLICATION:

This SOP outlines the policy and procedure for operation of the Bio-Rad Mini-Protean vertical electrophoresis system ("cell").

2.0 INTRODUCTION

The Mini-Protean cell is designed for fast and simple vertical electrophoresis using polyacrylamide gels. Precast Bio-Rad Ready Gels are recommended; handcast gels require a casting apparatus that we do not have in our lab. One or two gels can be run at a time.

3.0 SAFETY

Do not operate the Mini-Protean Cell at a voltage higher than 600 volts.
Before use, inspect the tank for cracks or chips, which may allow the buffer to leak from the tank and cause a potential electrical hazard. Additionally, inspect all electrical cables, banana jacks, and plugs for loose connections, cracks, breaks, or corrosion. Do not use any part that is cracked, charred, or corroded. These parts may also cause a potential electrical hazard. Contact your local Bio-Rad representative before using a part that may be considered hazardous.
During electrophoresis, inspect the base and workbench for any signs of buffer leakage.
If leaking buffer is detected, disconnect the power to the cell immediately and contact your local Bio-Rad representative.

4.0 SUMMARY OF METHOD

MATERIALS 1. Mini-Protean Cell (mini tank, assembly, and lid) 2. Precast polyacrylamide gel 3. 150V power supply 4. 1X gel running buffer 5. Sample loading buffer 6. Samples to be loaded 7. Micropipettor and gel loading tips **PART A: GEL PREPARATION AND ASSEMBLY** 1. Remove the Ready Gel from the storage pouch. 2. Gently remove the comb. Pull off the tape at the bottom of the Ready Gel cassette to expose the bottom edge of the gel. 3. Set the clamping frame to the open position on a clean flat surface. 4. Place the first gel sandwich or gel cassette (with the short plate facing inward) onto the gel supports; gel supports are molded into	

the bottom of the clamping frame assembly; there are two supports in each side of the assembly. 5. Now, place the second gel on the other side of the clamping frame, again by resting the gel onto the supports. 6. Using one hand, gently pull both gels towards each other, making sure that they rest firmly and squarely against the green gaskets that are built into the clamping frame; make certain that the short plates sit just below the notch at the top of the green gasket. 7. While gently squeezing the gel sandwiches or cassettes against the green gaskets with one hand (keeping constant pressure and both gels firmly held in place), slide the green arms of the clamping frame over the gels, locking them into place. 8. The arms of the clamping frame push the short plates of each gel cassette up against the notch in the green gasket, creating a leak-proof seal (check again to make certain that the short plates sit just below the notch at the top of the green gasket). At this point, the sample wells can be washed out with running buffer, and sample can be loaded.	It is critical that gel cassettes are placed into the clamping frame with the short plate facing inward. Also, the clamping frame requires 2 gels to create a functioning assembly. If an odd number of gels (1 or 3) is being run, you must use the buffer dam.
PART B: RUNNING THE GEL 1. Place the assembled gel(s) into the tank. The assembly should be inserted into the tank on the side away from you, with the black electrode on the left. 2. Pour 1X running buffer into the assembly (upper chamber). Before filling the rest of the tank, make sure the assembly does not leak. If it does, you must remove the assembly and assemble it correctly. 3. Fill the upper chamber with buffer until the level is just below the outer plate(s) of the gel(s). Fill the tank up to the 2-gel level marker (requires about 700 mL). 4. Make sure the wells are all filled with buffer. Use a pipet to blast out any stubborn air bubbles. This step also removes unpolymerized acrylamide from	The lid only goes on one way, so it is important to insert the assembly correctly. You do not want to have to move it after the gels are loaded with samples.

the wells, which can interfere with protein migration.	
5. Load samples and standards into the wells using a gel loading pipet tip or syringe. Be careful not to poke a hole in the gel or blast samples out when loading (stop just short of the second stop).	Be careful not to jostle or move the base after loading wells because you will lose your sample(s).
6. Place the lid on the base, matching the electrode colors.	
7. Connect the electrical leads to a power supply. Set to 200 volts and press start.	
8. Check the area around the electrodes to make sure tiny bubbles are forming. This is evidence of an electrical current in the buffer.	
9. Periodically check the system to make sure the tracking dye is migrating in the gel. Do not allow the tracking dye to migrate off your gel. Do not allow the buffer to heat above 40ºC or your gel could melt.	
10. When you are finished running the gel, stop the power supply and disconnect the leads.	
11. Remove the lid from the base and take out the assembly. Pour the buffer out before disassembling.	
12. Remove the gel from the assembly.	
13. Remove the gel from its cassette by separating the two plates of the cassette. This can be done easily by inserting a spatula between the plates and twisting.	
14. Remove the top plate and invert the other plate in a container of water, buffer, or staining solution. Agitate gently until the gel falls off the plate.	
15. Fix and/or stain the gels as desired.	
16. Visualize the gel using the appropriate light source (white light for Coomassie blue stain).	Be extremely careful with polyacrylamide gels, which are very fragile and rip easily.
17. Discard the electrophoresis buffer and gel.	
18. Rinse the lid, tank, and assembly with tap water and dry completely before storing.	

SOP ELC-004
EDVOTEK ELECTROPHORESIS CHAMBERS (M12 AND HEXAGEL)
Revision A, June 25, 2012

Title: Operation of Edvotek Horizontal Electrophoresis Chambers

1.0 SCOPE AND APPLICATION:

This SOP outlines the policy and procedure for operation of the Edvotek M12 and Hexagel horizontal electrophoresis chambers.

2.0 INTRODUCTION

The Edvotek system is designed for fast and simple horizontal electrophoresis using agarose gels. The gel is cast outside the base chamber using the included tray, combs, and bumpers for casting.

3.0 SAFETY

Do not operate the apparatus at a voltage higher than 300 volts.
Before use, inspect the box base for cracks or chips, which may allow the buffer to leak from the base and cause a potential electrical hazard. Additionally, inspect all electrical cables, and plugs for loose connections, breaks, or corrosion. Do not use any part that is cracked, charred, or corroded. These parts may also cause a potential electrical hazard. Contact your laboratory technician.
During electrophoresis, inspect the base and workbench for any signs of buffer leakage.
If leaking buffer is detected, disconnect the power to the cell immediately and contact your laboratory technician.

4.0 SUMMARY OF METHOD

MATERIALS
 	
1. Edvotek electrophoresis chamber (M12 or Hexagel)
2. Gel casting equipment
    1. Bumpers (2 per tray)
    2. Casting tray (7x7 cm or 7x14cm)
    3. Comb (6, 8 or 10 well)
3. Electrophoresis power supply
4. Agarose powder
5. 1X Running buffer such as TAE or TBE
6. Sample buffer/loading dye
7. Samples to be loaded
8. Micropipettor and tips	

PART A: GEL CASTING 1. Prepare 1X buffer and molten agarose. 2. Cool the molten agarose to 50-60ºC.	The 7x7cm casting trays hold approximately 30mL of molten agarose, and the 7x14cm hold about 60mL. **Warning:** Hot agarose (>60°C) may cause the

3. Press the black bumpers onto either side of the casting tray to form a tight seal. This is easiest if you place the bumper slit up on the bench and press the tray down onto it.	tray to warp or craze and will decrease the lifetime of the tray. Warping may also result in sample wells of uneven depth.
4. Select a comb (blue or white) with the appropriate number of wells, and insert it into the tray.	
5. Pour about 30mL of agarose into the 7x7 tray (60mL into the 7x14.) Check for bubbles or debris and remove these with a transfer pipet before the agarose cools.	
6. Allow 20-40 minutes for the agarose to cool and solidify.	
7. Carefully remove the comb and bumpers from the gel.	To avoid ripping the gel, it can be helpful to separate the gel from the bumpers and tray using a slim spatula before attempting to remove.
PART B: RUNNING THE GEL	
1. Place the casting tray with solidified gel into the electrophoresis chamber, orienting the wells toward the black electrode. The tab on the side of the tray will fit into a cut out on the side of the chamber.	
2. Pour 1X electrophoresis running buffer over the gel until it is covered by 2-6 mm.	
3. Make sure the wells are all filled with buffer. Use a pipet to blast out any stubborn air bubbles.	The M12 chamber requires about 400 mL buffer, while the Hexagel needs 2-3 times that amount.
4. Load samples and standards (premixed with sample buffer/loading dye to a final concentration of 1X) into the wells using a regular pipet tip. Be careful not to poke a hole in the gel or blast samples out when loading (stop just short of the second stop).	
5. Place the lid on the base, matching the electrode colors (black to black and red to red.)	
6. Connect the electrical leads to a power supply and choose an appropriate voltage, usually between 75 and 150 volts. Press start.	Be careful not to jostle or move the base after loading wells because the sample(s) can float out.
7. Check the area of buffer around the electrodes to make sure tiny bubbles are forming. This is evidence of electrical current passing through the buffer.	Edvotek DuoSource power supplies can be set to either 75 or 150 volts. Other power supplies can be adjusted to more specific voltages.

8. Periodically check the system to make sure the tracking dye (in the sample buffer) is migrating down the gel. Do not allow the tracking dye to migrate off your gel. Do not allow the buffer to heat above 40ºC or your gel could melt. If the chamber is very warm to the touch or excessively steamy, you may be overheating the system. 9. When you are finished running the gel, stop the power supply and disconnect the leads. 10. Remove the lid from the base. Carefully take out the gel in the tray and place it in a container for transport to the gel documentation system. 11. Discard the electrophoresis buffer and gel, treating as hazardous waste if necessary. 12. Rinse the lid, base, tray, comb, and bumpers with tap water and dry completely before storing. If agarose residue remains on any of the casting parts, wash gently with soapy water and a brush, rinse thoroughly, and dry.	Voltage is directly related to heat. If your gel is becoming hot, reduce the Voltage so as not to melt the gel. Avoid scrubbing or aggressively drying the inside of the chamber because the platinum electrodes are delicate and can be ripped out easily. A gentle tap water rinse and blot-dry with a paper towel is sufficient and will not damage the electrodes.

SOP GDS-004
CHEMIDOC IMAGER WITH IMAGE LAB SOFTWARE
Revision B, July 12, 2012

Title: Use of Bio-Rad ChemiDoc Imager Using Image Lab Software

1.0 SCOPE AND APPLICATION:

This SOP outlines the policy and procedure for use of the Bio-Rad ChemiDoc Imager with Image Lab software for gel documentation.

2.0 SUMMARY OF METHOD

MATERIALS 1. ChemiDoc Imager 2. Attached computer with Image Lab software 3. Gels to be imaged	Important: the Mitsubishi P93D thermal printer will only work on a computer using Windows XP. Windows 7 will not allow the printer driver to be installed. Image Lab works fine with Windows 7, but images will not print.
PART A: OPERATION	
1. Turn on the cabinet (switch is on the back left of the cabinet) and the camera power supply (black box).	
2. Double-click the Image Lab icon on the desktop to open the software.	
3. You may use an existing protocol by choosing Open under the File menu. Proceed to step 9.	
4. To create a new protocol, click New Protocol on the menu bar. Under Gel Imaging, select the application you need (nucleic acid or protein gel) and select the stain you are using.	
5. You may also select a gel type from a drop-down menu; this can be helpful if you are using the ChemiDoc to analyze your gel results. You may also enter the image area manually.	
6. You can select optimization for either faint or intense bands, or you can manually select the exposure time.	
7. You may also select the color you want the image displayed in, which should be the same as your stain. Please note, the printer will only print in black and white.	
8. If you would like to include an image analysis and/or a report in your protocol,	Another option is checking or unchecking the box next to “Highlight Saturated Pixels.” When checked, the display will show red highlights over pixels that are saturated (when the

check the boxes next to these and select parameters for Lane and Band Detection, Molecular Weight Determination, and the type of output for a report. These steps are optional. 9. When you are ready to run the protocol, click the yellow Position Gel button. Open the drawer on the cabinet and place your gel on either the UV light box or on top of the white conversion screen (if you are using white light illumination). For either UV or white light (with the conversion screen), you must have the filter in position 1. 10. Position the gel in the center of the box and close the drawer. You may zoom in or out by moving the camera meter below the image. When the image appears how you want it to, click the green Run Protocol button. This will initiate image collection. 11. After the exposure your image will appear in a new window. Go to File > Save to save the image. If you need to open the file in another program such as Microsoft Paint, you will need to export the file as a JPEG. Do this by going to File > Export > Displayed Image and save the image as a JPEG. 12. To print, go to File > Print > and click Print. Make sure the Mitsubishi P93D is selected as the printer. This is a thermal printer that uses glossy paper and prints small images. When the image has printed, pull against the cutting edge to detach your photo. 13. When you are finished with the ChemiDoc, wipe buffer and gel residue off the light box with a paper towel. Turn off the camera and the cabinet. **PART B: TROUBLESHOOTING GUIDE** 1. 1. If the ChemiDoc instrument is not detected by the software, make sure that BOTH the cabinet and the camera power supply are turned on. Lights should be visible on the front of the cabinet if it is on. 2. 2. If you see a bright circle on the image, check to make sure the flat field lens is not in position 2 of the filter wheel. This lens is white on top and pink on the bottom and is	camera was at its max light recording ability). These saturated pixels indicate a high level of fluorescence or intense color in that region of the gel. Unchecking the box will hide the highlights.

placed in position 2 during a calibration step. It should be removed after calibration, but if it was mistakenly left on, it can cause a circular reflection on gel images. 3. 3. If the printed images are an odd size or shape, make sure the print size is 1280x1280. Go to File > Print > Page Setup and choose 1280x1280 as the page size.	

SOP GWC-001
CLEANING GLASSWARE
Revision B, July 12, 2012

Title: Cleaning Glassware

1.0 SCOPE AND APPLICATION:

This SOP outlines the policy and procedure for cleaning used glassware and plasticware.

2.0 SUMMARY OF METHOD

PROCEDURE	**COMMENTS**
1. Remove any tape or labels from the glassware and remove any pen marks with acetone or ethanol.	Use of this SOP indicates that the user has disposed properly of any hazardous materials contained in the dirty glassware.
2. Fill a wash tub with warm tap water and a few mL of Micro-90 detergent.	Use a flat razor scraper to remove tape and labels, if necessary.
3. Soak dirty glassware for a few minutes before scrubbing thoroughly with a brush.	
4. Rinse thoroughly with tap water three times.	If a stubborn chemical residue persists, soak the glassware in tap water and use a brush or a spatula to remove the residue (only if **nonhazardous**). Glassware with persistent residue of a **hazardous** chemical should be marked as hazardous waste (be sure to name the chemical).
5. Rinse thoroughly with deionized water three times.	
6. To dry, hang glassware on pegboard if possible, or place upside down over paper towels or Labmat.	

SOP MCP-001
USE OF VWR MICROPIPETTORS P10, P20, P100, P200 AND P1000
Revision A, August 15, 2012

Title: Operation and maintenance of VWR Micropipettors

1.0 SCOPE AND APPLICATION:

This SOP outlines the procedure for operation and maintenance of VWR P10, P20, P100, P200 and P1000 micropipettors.

2.0 SUMMARY OF METHOD

PART A: OPERATION	**COMMENTS**
1. Each student or group should check out a set of micropipettors for the duration of the course. Your instructor will tell you how to record your selection and if you need to validate your set (see Part C). If any micropipettors in your set are damaged, notify your instructor immediately.	Check-out will prevent abuse of micropipettors, and ensure that errors in measurement can be tracked to an out-of-calibration instrument. Note that the most common measuring range for a P10 is 1.0-10.0µL, for P20, 2.0-20.0 µL, for P100, 10.0-100.0 µL, for P200, 20.0-200.0 µL, for P1000, 100.0-1000.0 µL. **Never** adjust a micropipettor outside of this range!
2. Select a micropipettor from your set that will measure the volume you need.	On P10, P20, P100, and P200 micropipettors, the black digits indicate microliters; the red digits indicate tenths and hundredths of microliters. On P1000 micropipettors, the red digits indicates milliliters and the black digits indicate microliters. The appearance of the minimum and maximum volumes on the analog readout of each size micropipettor are shown in the appendix to this SOP.
3. Set volume using volume adjustment knob. Hold micropipettor in one hand, and with other hand, turn volume adjustment knob counterclockwise so volume indicator is 1/3 revolution above desired setting, then turn slowly clockwise until indicator shows desired volume. If micropipettor dial is past either its high or low limits, or the dial will not rotate, notify your instructor immediately.	Always dial down to the volume setting. This prevents mechanical back lash from affecting accuracy. Otherwise, precision and accuracy will be affected.
4. If you pass the desired setting, turn dial 1/3 revolution higher than desired and reset volume.	Do not touch the tips. To avoid contamination a fresh tip should be used for each measurement.
5. Attach a new disposable tip to the pipette shaft by pressing shaft into tip while tip is still in box. Press only hard enough to make a positive airtight seal.	Never immerse the tip more than a few millimeters below the surface of the fluid. A deeper immersion can cause fluid to cling to outside of tip, delivering more fluid than was desired.
6. Press plunger to first stop. This part of the stroke is volume indicator.	
7. Holding micropipettor vertically (perpendicular to the bench), immerse the last few millimeters of the disposable tip in the fluid to be measured.	Never let the plunger snap up. This can cause air bubbles to fill the tip, destroying accuracy.

8. Release the plunger slowly until it has returned to its original position.	
9. Pause for a few seconds to ensure that the full volume of fluid is drawn into the tip.	Always keep the micropipettor in vertical position to ensure that no liquids enter the micropipetter or drip from the disposable tip.
10. Withdraw the tip from the sample, keeping the micropipettor completely vertical.	
11. Inspect tip to make sure there is no air bubble on the inside and there is no solution on the outside of the tip.	
12. If there is an air bubble inside tip, you must pipet this volume back into the sample and measure again, repeating steps 5-10.	
13. If there is solution on the outside of the tip, touch tip to the wall of the solution container to remove it.	
14. To dispense the sample touch the tip end against the side wall of the receiving vessel and depress the plunger slowly to the first stop, then press the plunger to the second stop, expelling any residual liquid in the tip.	Dispense the fluid into the lowest possible place in the receiving vessel.
15. With the plunger fully pressed, withdraw pipette from the vessel carefully, with the tip remaining against the wall of the vessel.	
16. Release the plunger to return to the up position.	
17. Discard the tip into a waste beaker or trash receptacle by depressing the tip ejector button.	
	Never discard tips into sinks. Always use a waste beaker or trash receptacle.
PART B: TIP SELECTION	If you measure a **hazardous material** (chemical or biological), dispose of contaminated tips into a properly labeled container and store with the hazardous waste.
1. Tips must seal properly on the shaft to assure an airtight seal and avoid leaks or poor accuracy.	
2. Tips must be soft and flexible so that the shaft is not scratched or worn prematurely.	Barrier tips provide the best protection from air contamination, but they do not always fit on the smallest micropipettors.
3. Tips must be free from microscopic particles.	Do not use sterile tips for non-sterile applications. Do not use gel loading tips for non-gel-loading applications. These tips are expensive.
4. The tip orifice must be the correct size, and orifice size and geometry must be consistent from tip to tip.	

5. Interior and exterior surfaces must be clear, smooth and hydrophobic to avoid retention of liquid. **PART C: VALIDATION** 1. Micropipettes should be validated at least once a semester. 2. Obtain a printed copy of the blank Micropipettor Validation Log (Appendix). Record the serial numbers and set number of your micropipettor set. Also include the names of people in your group. 3. Locate an analytical balance and calibrate if necessary. 4. Dial a micropipettor to its highest volume. 5. Place a weigh boat on the weighing pan of the balance, close the draft shield, and tare the balance. 6. When balance is stable, measure highest possible volume of purified water with the micropipettor, and dispense into the weigh boat in the balance. 7. Close the draft shield and wait for the balance to stabilize. Record the mass of the water in the validation log. 8. Repeat steps 6 and 7, adding each measurement of water to the weigh boat and recording the total mass of water with each addition. 9. Make ten measurements. Discard the weight boat with water. 10. Repeat steps 4 through 9 with each of the other micropipettors in your set. 11. When complete, enter the data into the Excel file, **Micropipettor Validation Log Template**. 12. Save and print or email the file to your instructor.	Your instructor will tell you if this process has been assigned to your class. Functions programmed in the workbook will automatically calculate the volume delivered in each measurement, and the resulting percent precision.

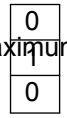
APPENDIX TO SOP MCP-001
USE OF VWR MICROPIPETTORS P10, P20, P100, P200 AND P1000

Updated May 19, 2006

Appearance of Analog Readout at Minimum and Maximum Volumes of Micropipettors

P1000 (100-1000 µL)

Minimum, 100 µL

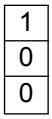


Maximum, 1000 µL

P1000 (200-1000 µL)

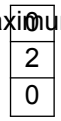
Minimum, 200 µL

Maximum, 1000 µL

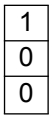


P200 (20-200 µL)

Minimum, 20 µL



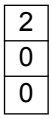
Maximum, 200 µL



P200 (50-200 µL)

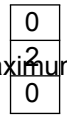
Minimum, 50 µL

Maximum, 200 µL

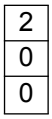


P20 (2-20 µL)

Minimum, 2 µL



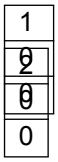
Maximum, 20 µL



P10 (1-10 µL)

Minimum, 1 µL

Maximum, 10 µL



SOP PCR-004
APPLIED BIOSYSTEMS 2720 THERMAL CYCLER
Revision A, February 22, 2011

Title: Operation of Applied Biosystems 2720 Thermal Cycler

1.0 SCOPE AND APPLICATION:

This SOP outlines the policy and procedure for operation of the AB 2720 thermal cycler.

2.0 SUMMARY OF METHOD

MATERIALS • AB 2720 thermal cycler • PCR reaction tubes, strips, or 96-well plate **PART A: OPERATION**	
1. Plug in the thermal cycler and turn it on. You will see a Home screen with the time, date, and temperature at the top and RUN, CREATE, EDIT, UTIL, and USER at the bottom.	
2. Use the F1-F5 softkeys to select one of the options at the bottom of the screen: a. F1 to RUN an existing protocol b. F2 to CREATE a new protocol c. F3 to EDIT and existing protocol	
3. Run a protocol that is already saved. Choose the protocol you want with the arrow keys and hit the softkey under VIEW to check the program. If the program is correct, hit the softkey under START to begin. You must enter the volume of your reaction mixture before the program will begin. At this step, open the lid and insert your reaction tubes, strips, or plate into the sample block, close the lid, and proceed.	IMPORTANT: this instrument will only hold 0.2 mL sample tubes. If your reaction is in a 0.5 mL tube, you MUST transfer it to a 0.2 mL tube.
4. Create a new protocol. Use the arrow keys to move through the screen and use the number keys to enter the appropriate times and temperatures. When you are finished you may SAVE or START.	
5. Edit an existing protocol. Choose the protocol you want with the arrow keys and hit the softkey under VIEW to edit. Make the necessary changes and SAVE.	
6. When you have started a protocol the	

instrument will warm up to the start temp and then begin the program. There is no pause before the program starts, as there is with some other instruments, so make sure your samples are in the block before starting. 7. Every protocol has an automatic hold at 4 degrees at the end of the protocol, so your samples will be chilled after the run. Still, you should attempt to remove them as soon as possible after the run and freeze them.	

SOP PHM-002
OPERATION AND MAINTENANCE OF ACCUMET MODEL AB15 pH METER
Revision A, June 8, 2011

Title: Operation and standardization of AB15 pH meter

1.0 SCOPE AND APPLICATION:

This SOP outlines the procedure for the proper operation and standardization of the AB15 pH meter.

2.0 SUMMARY OF METHOD

MATERIALS	**COMMENTS**
• AB15 pH meter • pH 4, 7, and 10 standard buffer solutions (if calibrating) • 50-mL beakers • pH probe with electrode • Deionized water in wash bottle • Waste beaker (250-500 ml) • Labeling tape and sharpie	
PART A: STANDARDIZATION	
1. Turn on pH meter and allow it to warm up for at least 5 minutes. The probe must be supported by the holder.	The tip of the probe should be immersed in a storage solution but not touching the bottom of the vessel (this can damage the probe).
2. Select two standards that bracket the expected pH of the solution to be measured. If basic, use standards of pH 7 and 10. If acidic, use standards of pH 4 and 7. If the expected pH is unknown, use standards of pH 4, 7, and 10.	Do not keep a pH probe out of a solution for longer than a minute; it may damage the probe. Do not turn probe upside down.
3. Label 50-mL beakers with the pH of the selected calibration standards. Pour 25-35 ml of standard buffers into the appropriately labeled beakers.	
4. Check that the pH meter is in pH mode (on the top left of screen); if not, press and release the mode key until the meter is in pH mode.	The tip of the probe should not be touching any surface.
5. Completely rinse the probe with deionized water (over the waste collection beaker) and immerse the electrode 1 cm in a buffer. Wait for the reading to stabilize, and if it is greater than ±0.03 pH units from the true pH value, then you must standardize the meter. If not, proceed to PART B.	

6. Press the **setup** key twice and then the **enter** key to clear the existing calibration. 7. Press the **std** key to access Standardization mode. The selected buffer group will appear (2, 4, 7, 10, 12). 8. Press **std** again to initiate standardization. The meter will recognize the buffer and flash the pH value on the screen. When the stable icon appears, proceed to step 9. 9. Completely rinse the probe with deionized water (over the waste collection beaker) and immerse the electrode 1 cm in the other buffer. 10. Repeat steps 7, 8, and 9 with this buffer and a third if necessary. 11. Completely rinse the probe with deionized water (over the waste collection beaker), and immerse the tip of the electrode into a storage solution in a 50 mL Erlenmeyer flask. **PART B: OPERATION** 1. Check that the meter is in pH mode (on the top left of the display) and if it is not, press and release the **MODE** key until pH appears on the display. 2. Completely rinse the probe with deionized water (over the waste collection beaker) and immerse the electrode 1 cm in the sample. Wait for the reading to stabilize. 3. Record the pH measurement and remove the probe from the sample. Rinse thoroughly with deionized water (over the waste collection beaker) and replace in the storage solution.	When the meter accepts the second buffer, it will display the percent slope associated with the electrode's performance prior to returning to the measure mode. If the electrode is with in the range of 90-102%, the **GOOD ELECTRODE** message will appear. If the electrode is outside this range, the meter will display **ELECTRODE ERROR** message and will not return to the measure screen until the user presses the enter key. Add any comments, such as an error message on the meter, low fill solution in the probe, or low or contaminated probe storage solution, to the appropriate log sheet found in the equipment log book. The meter will permit the user to use an electrode outside the recommended range, but the **ERROR** message will still show. Try recalibration (steps 6-10), if you get an **ELECTRODE ERROR** message. If you get this error message again after the second calibration, report the electrode failure immediately to your supervisor and enter this on the instrument log sheet.

APPENDIX TO SOP PHM-002
pH PROBES FOR THE ACCUMET MODEL AB15 pH METER

The selection of the appropriate pH electrode (probe) to use with a pH meter can have an impact on the quality of measurements and on the life of the equipment.

As of May 14, 2010, we are using **Fisher Scientific accumet Liquid-Filled Mercury-Free pH/ATC Epoxy Body Combination Electrodes** (catalog# 13-620-631) with our pH meters. These have a double junction with an Ag/AgCl electrode. They can be filled with saturated KCl solution (catalog# SP138-500).

These electrodes are mercury-free, refillable, and are safe to use with Tris.

You may also see **VWR SympHony refillable calomel electrodes** (catalog# 14002-772). They can be filled with calomel fill solution (catalog# 34108-024).

SOP SOL-001
LABELING SOLUTIONS
Revision A, January 17, 2008

Title: Labeling Prepared Solutions

1.0 SCOPE AND APPLICATION:

This SOP outlines the policy and procedure for labeling solutions prepared by students, faculty and staff.

2.0 SUMMARY OF METHOD

	COMMENTS
1. Obtain a solution prep form from the instructor or from the lab form files.	
2. Complete any calculations and enter them at the bottom of the solution prep form (or reference a page in your lab protocol).	
3. Obtain all chemicals, glassware, and equipment needed to prepare the solution and assemble so they are all within reach.	
4. Make a label for the vessel in which you will prepare the solution; use whatever name you would usually call the solution (for example, "1X TAE buffer"). Leave plenty of room for information that will be added later. Affix the label to the vessel before adding any chemical.	Any solution or chemical must be labeled with its identity if it is removed from its primary container (i.e. the stock bottle).
5. Think of a unique ID number (control number) for the solution. The format of control numbers in our labs should follow the following example: TAE-EG-041106 The first three letters are an abbreviation of the common name of the solution (in this example, Tris-Acetate-EDTA buffer). The second two letters are the initials of the person (or one of the people) who prepared the solution (Evelyn Goss). The last six digits are the date (always YYMMDD; in this example, November 6, 2004).	The purpose of control numbers is for any person working in the lab to discriminate between any two solutions, regardless of how similar they are, by the control number. In the unusual case that one individual makes the same solution more than once in one day, simply insert numbers after the first three letters to indicate the order in which they were made. For example, the second batch of TAE buffer that Evelyn Goss made on November 6, 2004 would have the control number: TAE-2-EG-041106.
6. Fill out all sections of the solution prep form (see Appendix A for an example). If a field contains information that is not applicable, enter "N/A" in that field. Enter all information (except the amount of each	

chemical used) before preparing the solution. Enter the amounts used after weighing and delivering the chemical(s) to the preparation vessel. 7. After preparing the solution and transferring solution and label to the storage vessel (if necessary), complete the label by including the following information: Solution prep control number Any special instructions, such as expiration date or storage conditions Safety warnings if the solution contains hazardous materials Course number and semester (if applicable) 8. File the solution prep form alphabetically in the top drawer of the black file cabinet. If you are a student taking a course, hand into your instructor, who will make copies as needed and give the original back to you for your lab report. The instructor should always keep a copy on file in the lab.	Never enter the amount of a chemical used until you have actually measured and added the quantity. Do not enter the calculated mass or volume; only enter the actual, measured quantity.

Appendix A

SOLUTION PREP FORM

Control # TAE-LG-06Nov2004

Name of Solution/Media: 50X TAE Buffer

Amount prepared: 1 L Preparation Date: November 6, 2004

Preparer(s): Lab Student

Component	**Brand/lot # (Vendor)**	**Storage conditions/ date received**	**FW or initial concentration**	**Amount used**	**Final concentration**
Trizma base	Sigma/022385	15May2003	121.1 g/mol	242 g	2 M
Glacial acetic acid	EMD/43DBB31	5March1999	60.05 g/mol	57.1 mL	5.71%
EDTA	Fisher/ 960654A	25Jan2002	372.24 g/mol	37.2 g	100 mM

Balance used #5	Calibration status L.S. calibrated 6Nov2004 – OK	
pH meter used not used	Calibration status Not calibrated	
Initial pH Not measured	Final pH Not measured	Adjusted pH with Not measured
Prep temperature 22.3°C	Sterilization procedure/ sterility testing Not sterilized	Storage conditions Room 3121.03, student refrigerator, 1414 shelf

Calculations/Comments:

Trizma base: 1 L x 2 mol/L x 121.1 g/mol = 242.2 g

EDTA: 1 L x 100 mmol/L x 1 mol/1000 mmol x 372.24 g/mol = 37.224 g

SOP SPC-004
LAMBDA BIO+ SPECTROPHOTOMETER
Revision A, June 8, 2011

Title: Use and Maintenance of the Lambda Bio+ Spectrophotometer

1.0 SCOPE AND APPLICATION:

This SOP outlines the procedure for use and maintenance of the Lambda Bio+ Spectrophotometer.

2.0 SUMMARY OF METHOD

MATERIALS • Lambda Bio+ Spectrophotometer • Cuvettes with 15 mm sample window	
NOTE: The beam height of this instrument is 15 mm. You must either fill the cuvette at least 15 mm high, or use a 15 mm height adaptor. When using a UV wavelength, you must use UV-transparent cuvettes.	Some plastics are not UV-transparent and would not be useful for UV absorbance readings. Quartz cuvettes are UV-transparent. Make sure that the sides of the cuvette facing the sides of the instrument are optically transparent. The light path travels from right to left across the instrument. It is best to handle cuvettes by the optically opaque sides to avoid leaving any residue on the clear sides.
PART A: SINGLE WAVELENGTH READINGS 1. Turn on the spectrophotometer by pressing the power button. 2. Wait for the instrument to initialize and complete internal tests. The default home page will be displayed. 3. Press 1 on the number keypad to choose Single Wavelength. 4. Enter the wavelength you would like to use, then press the green ► button on the keypad to accept. 5. Insert a cuvette containing the appropriate blank solution. Press the **0A/100%T** button on the keypad to take a blank reading. 6. Remove the blank cuvette and insert a cuvette containing the sample. Press the green ► button to take a reading. The absorbance will be displayed. 7. If the absorbance value of a sample is above the range of the instrument, you will need to dilute the sample. 8. Repeat with any other samples. 9. When you are finished, clean the cuvettes by first emptying them, then flushing with several changes of purified water. Next, flush with several changes of 70-95% ethanol. Finally, dry upside down on a paper towel before storing in a safe place.	The blank solution should be identical to or as similar as possible to the solution in which the compound to be measured is suspended. This is usually water or an aqueous buffer. A 1/10 dilution is a good place to start. Use the blank solution to dilute the sample. If the absorbance is still too high, dilute again and repeat until an accurate measurement is obtained. Then, multiply the absorbance reading by the dilution factor to obtain the absorbance of the original concentration.

10. If you are the last to use the spectrophotometer, power off the instrument.	**Never use any abrasive cleansers, soaps, or brushes on cuvettes**. This can scratch them or leave a contaminating residue.
PART B: MULTI-WAVELENGTH READINGS	Disposable plastic cuvettes can be used a few times before they become scratched or stained and need to be discarded. Clean these carefully and they will last for several uses.
1. Follow the procedure for single wavelength readings with these two modifications:	Quartz cuvettes look like glass and are made to have a long life. Treat these with special care because they are very expensive. They will last many years if used carefully.
2. In Step 3, choose 2 for multi-wavelength readings.	
3. In Step 4, enter two or more wavelengths.	
PART C: ABSORBANCE SPECTRUM	
1. Choose 3 for Spectrum in the default home page.	
2. Enter the start and end wavelengths and press the green ► button.	
3. Insert a cuvette containing the appropriate blank solution. Press the **0A/100%T** button on the keypad to take a blank reading.	
4. Remove the blank cuvette and insert a cuvette containing the sample. Press the green ► button to take a reading. The absorbance spectrum will be displayed.	
5. Repeat with any other samples.	
6. When you are finished, clean the cuvettes by first emptying them, then flushing with several changes of purified water. Next, flush with several changes of 70-95% ethanol. Finally, dry upside down on a paper towel before storing in a safe place.	The blank solution should be identical to or as similar as possible to the solution in which the compound to be measured is suspended. This is usually water or an aqueous buffer.
7. If you are the last to use the spectrophotometer, power off the instrument.	
PART C: KINETICS	
1. From the Standard Methods home screen, press 6 for Kinetics.	A 1/10 dilution is a good place to start. Use the blank solution to dilute the sample. If the absorbance is still too high, dilute again and repeat until an accurate measurement is obtained. Then, multiply the absorbance reading by the dilution factor to obtain the absorbance of the original concentration.
2. Enter the wavelength, delay time (if any), duration time, and interval length using the numeric keypad and arrows.	
3. Press the green ► button to continue.	**Never use any abrasive cleansers, soaps, or brushes on cuvettes**. This can scratch them or leave a contaminating residue.
4. Select the measurement mode using the left and right arrows (you will most likely want to select “Slope”). Press the down arrow to get to units, then enter the units manually or press the Options button	Disposable plastic cuvettes can be used a few times before they become scratched or

to choose from a list of units. Press the down arrow and change the “Factor” setting if you need to multiply results by a dilution factor. 5. Press the green ► button to continue. 6. Insert a cuvette containing the reference sample and press the **0A/100%T** button to take a blank reading. 7. Insert a cuvette containing the sample and press the green ► button to begin measuring. The window will display a real-time plot of absorbance. 8. Press the Options button to print, save, or change view settings.	stained and need to be discarded. Clean these carefully and they will last for several uses. Quartz cuvettes look like glass and are made to have a long life. Treat these with special care because they are very expensive. They will last many years if used carefully.

SOP SPC-008
NANODROP 2000 SPECTROPHOTOMETER
Revision A, June 8, 2011

Title: Operation of the NanoDrop 2000 Spectrophotometer

1.0 SCOPE AND APPLICATION:

This SOP outlines the policy and procedure for operation of the NanoDrop 2000 Spectrophotometer.

2.0 SUMMARY OF METHOD

MATERIALS • Nanodrop 2000 Spectrophotometer • Attached computer with ND2000 software • P2 or P10 pipettor and tips • Deionized water and Kimwipes **PART A: Using the NanoDrop 2000** 1. Open the ND2000 software on the desktop of the attached computer. If asked for a password, click Enter without typing a password. 2. The main menu will open and you will be able to choose from a number of methods, including Nucleic Acid, Protein, etc. Select the appropriate method and it will open. 3. You will be asked if you would like to open the previously used workbook; choose No. 4. Make sure arm is down and click ok to start the Wavelength Verification. 5. Go to File and New Workbook. Name and save your workbook; all samples will now be saved to your workbook until you select a new workbook. This provides a simple way to tabulate and report multiple measurements. 6. Establish a reference or blank using the appropriate buffer or water (whatever your sample is dissolved in). Pipette 1-2uL of blank solution onto the bottom pedestal and click “BLANK”, located at the top left corner of the screen. 7. Wipe upper and lower pedestals with a Kimwipe. 8. Pipette 1-2uL of the sample onto the bottom pedestal and click “MEASURE” to obtain an absorbance. You may name the sample in the Sample ID field.	**Note: the blank reading will not display any values or a spectrum. These will not appear until you perform a measurement. If you blank again after taking a measurement, the data and spectrum from the previous measurement will remain on the screen.** **To verify the blank, click Measure to record your blank solution as a sample. A spectrum and absorbance and concentration values will appear on the display.**

9. Wipe upper and lower pedestals with a Kimwipe. 10. Measure other samples by repeating steps 8 and 9. 11. If desired, pipette 1-2uL of deionized water onto the bottom pedestal and wipe with a Kimwipe to clean the sample holder.	**DO NOT use detergents or alcohol to clean the pedestals, as this may result in pedestals becoming unconditioned.**

Record of Revisions to Basic Equipment Standard Operating Procedures

Editor's Note from Fall 2019: This book of basic equipment standard operating procedures was created to simplify the former "SOP Book" into a more helpful guide for students in 1414 and 1415. The old SOP book contained SOPs for too many advanced instruments that were not usable for first-year students, and which sometimes conflicted with lab manual instructions for second-year students.

Table 1: Updates in the 1st Edition, Fall 2019

SOP Title	*Changes*	*Justification*	*Name of Editor*