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A Manual for a Junior Lab Technician

Jared Blaine Burton
Utah State University

A Manual For A Junior Lab Technician

by

Jared Blaine Burton

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Dear New Junior Lab Technician,

Welcome to Dr. Barnard's laboratory. My name is Jared Burton, and I am currently in medical school. I wanted to write a little bit to give you a "heads-up" about what you should expect working here. I worked in this lab for three years. I picked up quite a bit of experience and research time during my employment here. It was a good, on-campus job. I thought I would write a little manual to be kept in the lab, with the hopes that some future employee will benefit from it, and not be quite as lost as I was when I first began.

When I was first taken on as a junior research technician, I didn't have much information given me. I was mainly listening to and observing my supervisor and taking copious notes. Much of my experience came through trial and error, as I am sure much of yours will, too. However, it is my hope that this manual will provide you with much of the information you need not only to begin your job here on the right foot, but also to assist you in staying on track and to help you to get as much knowledge and experience as possible from this lab experience. If you let it, the job will be a valuable learning experience.

Sincerely,

Jared Burton

Background rules:

- Do not bring food or drink into the lab.
- Keep your personal belongings (backpack, coat, etc) within the designated area located by the door.
- Do not wear shorts or sandals into the lab. Wear long pants and shoes at all times.
- It is recommended that you wear glasses as opposed to contact lenses in the lab. If you choose to wear contact lenses, please wear goggles if there is even the smallest indication that you should have eye protection.
- If there are injuries (even minor), spills, or other accidents, report them to the lab supervisor immediately. (See the official laboratory procedures manual for more information.)
- Remember to fill out a time card monthly… (as if that needed to be said).

General Information:

Major cell types (and control drug for each):

- A-549 (HPMPC @ 320 ug/ml)
- BSC-1 (Ribavirin @ 1000 ug/ml)
- CV-1 (Ribavirin @ 1000 ug/ml)
- KB (Pirodavir @ 3.2 or 10 ug/ml- ask supervisor)
- LLC-MK2 (Ribavirin @ 1000ug/ml)
- MA104 (Ribavirin @ 1000 ug/ml)

Major viruses, and which cell type they are tested in, with control drug:

- Ad A-549 (HPMPC @ 320 ug/ml)
- Me CV-1 (Ribavirin @ 1000 ug/ml)
- PIV MA104 (Ribavirin @ 1000 ug/ml)
- PCV BSC-1 (Ribavirin @ 1000 ug/ml)
- PT LLC-MK2 (Ribavirin @ 1000ug/ml)
- RSV MA104 (Ribavirin @ 1000 ug/ml)
- RV-2 KB (Pirodavir @ 3.2 or 10 ug/ml)

Antiviral Testing:

Labeling Plates:

Learn what viruses will be tested (and subsequently refer to the above information to find out what cells (in plates already prepared by the supervisor or yourself) are needed for that specific virus), in what format (4- or 8-dilution), and at what concentration. Find out what drugs will be tested with each virus. Remove the plates from the incubator in the cell room. Write on the plates (of the correct cell line) the drugs to be tested on that plate and at what concentration. (If it is to be a 4-dilution test, then only 4 drugs per plate, if an 8-dilution test, then only 2 drugs per plate.) Return the plates to the incubator.

Drugs:

Obtain the list of what drugs you will be testing. Find the drugs- fresh (powder) and frozen, in Rm. 308. Get out the sheet for each drug, found in the file cabinets, in Rm. 308A. Get out snap-cap tubes to put drugs in, and label them with the drug (both ARB and NR numbers), and the solubility. Check the balance to see if truly zeroed before supervisor weighs the drug. Record weight

on the tube and on the specific drug sheet (also, in addition to the weight, record the date, your initials, and the purpose of the drug)

Adding the drug:

Normally, the supervisor will perform this part of the experiment. She will advise.

Adding the virus:

Obtain a list of the viruses to be used, and at what concentration. Also, find out how much of each virus should be added to the media you will be using. Look up each virus in the virus inventory and mark off the number of vials generally needed (normally you will only need one vial). Unlock the freezer and find the virus, pull it out, and either keep it frozen in a safe place until you need it (if supervisor is not ready) or bring it to the virus room. Record in the virus inventory book by the freezer what virus you removed, when, and by whom. Proceed to the virus room and place the virus vials in the water bath (at 37 degrees Celsius). Get out the troughs (from under the hood) in which to put media. The number of troughs needed is equal to the number of viruses you are using, plus a cell control trough (with only media and no virus). Using the list you obtained from your supervisor, label each trough with the virus that you will add to it (and label one "cell control"). Fill the troughs for the viruses with the correct amount of MEM 4% in each, and fill the cell control trough (use a little over half the total amount of media used in the rest of the troughs combined). Remove the virus vials from the water bath and spray copiously with ethanol and vortex for a few seconds. Pipette the correct amount of each virus (as per the list from your supervisor) into the appropriate trough. When your supervisor brings in the plates, run them through the diluting machine in the virus hood (ask your supervisor for specific information about the programming of this machine).

When the plates are diluted, to each plate add media (without any virus) to the outer two columns on each side, and to the three top wells in the columns 6 and 7 (for the plate's cell control). Then add the appropriate virus (from the appropriate trough) to columns 3,4,5,8,9, and 10 of the respective plate. Use an 8-channel pipetter for this process- making sure to change the tips in between viruses, so as to not contaminate any plate with a virus other then one to be tested on it. Then, label the plates on the side with the virus, the virus #, the virus concentration, and the date. Put the plates in the incubator in the main room. Most plated should be stored at 37 degrees Celsius. RV plates should be stored at 33 degrees.

Sheets:

These should be done weekly, preferably each Friday. Fill out the virus sheets on the computer, using the information you will find on the plates (the cell type, the passage number, the virus, the virus number, the virus concentration, the drugs used on the plate, and the concentration of each drug). If it is a 4-dilution plate (meaning there can be four drugs on a plate), the concentration down the side of each plate, for each drug, will be reduced by a power of ten. (For example, if the plate has a drug written on it with a concentration of 10 ug/ml, then on the computer you will write, along the side going down, 10, then 1.0, then 0.1, and finally 0.01.) If it is an 8-dilution plate, the concentration will decrease by half logs. (For example, if the original concentration is 1000 ug/ml, then you will write 1000, 320, 100, 32, 10, 3.2, 1.0, and 0.32.) In order to complete the sheets you will also need to use your supervisor's notes (from her lab book) to determine what each drug was soluble in- usually WFI or DMSO, and if it precipitated of created a suspension when mixed together. Save a sheet for each plate under the title: virus, virus #, drug 1,2,3 and 4. Save the sheets from a

given week in a folder (on the computer's desktop) with a title of the dates of that week.

Virus Titrations:

Determine what virus is to be titrated. Determine what cell line that virus is in and obtain the correct plate from the cell incubator. On it, mark it with lines so there are columns of four, usually drawn the long way. Draw a line to separate the last row from the rest, for this will be the cell control. Label each group of four at the top with the correct virus name and number that will be titrated on it. Also, write the date on the front of the plate. Next, remove the virus from the freezer and mark it off on the virus inventory and write it in the virus inventory book near the freezer. Place it in the water bath until thawed, then spray it with ethanol and vortex it. In the virus hood and using a micropipetter, add 10 uL of the virus to the top well of each column. Then, discard the vial of virus. Run the plate through the diluting machine (after programming supervision from your supervisor), making sure to change the tips after each dilution on each row. Then place the plate in the incubator in the main room (at 37 degrees Celsius, unless it is RV, then at 33 degrees Celsius).

Autoclaving:

Trash bags:

There are special trash receptacles in the cell room, in the virus room, and under the microscope. These are the ones with the red biohazard bags in them. It is your responsibility to empty them when they are full. You will put on gloves, then remove the bag, tie it, and place it in the tray to be autoclaved. Replace the receptacle with a new biohazard bag. The used bags should be autoclaved for thirty minutes on the fast cycle before being thrown in the regular dumpster. This makes certain that all virus and potentially hazardous items are destroyed.

Pipettes:

Used pipettes will be in the tan trays on the side of each hood (virus and cell). When these trays are full, put them in the autoclave tray and autoclave them for 20 minutes on the fast cycle. When through, put them in the empty pipette boxes kept under the cell hood, close them with tape, and throw them in the dumpster. (Be sure to throw away the pipettes only in enclosed boxes.) Then clean the tan trays with soap and water and allow them to dry. Once dry, put on in the virus room (if there is not one there still) and put the rest in the cell room stacked on the side of the hood.

Flasks from cell and virus rooms:

When the large flask from either hood is nearly full, either your supervisor or you will need to remove it and replace it with a clean one (found in the clean dishes area). Make sure the connections from the vacuum pump to the clean flask are tight and that it is sealed completely. The full flask needs to be placed in the tray for autoclaving. Fill the tray with water so the bottom is covered. Place tin foil over the openings at the top- tight enough to stay on, yet loose enough as to not allow the high pressures of the autoclave to blow it off. Place it in the autoclave for thirty minutes on the liquid cycle. When through, empty the contents down the sink and wash the flask with the wire, long-handled brush, and allow to air dry beside the sink.

Tips:

See **Tips**.

Cell Culture:

Passing:

You will most likely be required to carry a few cell lines. Each cell line is somewhat different, but there are some guidelines and procedures we use in the

lab for each of them. This is typically done at the first of the week. First of all, turn on the cell hood and spray it with ethanol and wipe it down. Wear gloves and spray them with ethanol as well. Remove the cells (one cell line at a time) from the incubator and check them under the microscope to make sure they are confluent. Then, if so, remove the trypsin and the appropriate growth media for your cell line (ask supervisor) from the fridge. Prepare extra flasks that you will put the cells into after they are passed. (Usually two new flasks per flasks.) Loosen the lids to the trypsin and the cell flask. Open an aspiration pipette and suck out the media from the flask. Add 1 mL of trypsin to the flask, rinse it down the sides 5-6 times, and then remove it. Add 2 mL of trypsin and wash it sown the sides 2-3 times. Place it in the incubator. (The time needed here depends greatly upon the cell line. It can range from 1 minute to about 20 minutes.) When the cells are ready (meaning they are beginning to pull apart and you can see holes in the cell sheet on the bottom of the flask), tap the flask hard, to spread and loosen the cells. Add 8 mL of the growth media and wash it around, mixing and removing cells from the sides as you do so. Pipette the fluid up and transfer some to the new flasks at the ratio given you by your supervisor. (Each cell line is passed at a different ratio.) In each new flask with cells in it, add enough of the growth media to equal 12 mL. Then tighten the lids, place the flasks in the incubator, and put the rest of the containers away in the fridge. Clean the hood by spraying it and the vacuum hose with ethanol, and subsequently wiping it down. Turn off the vacuum and the hood. After a few hours, loosen the lids of your flasks to let the pressure escape, and tighten them back up again.

Changing Media:

On Thursdays, take your flasks and suck out all the media. Then add 12 mL of growth media to the flask and return it to the incubator. (This will rid the flask of the waste products in the media and provide the cells nourishment from the fresh media.)

Cleaning:

Keep lab tidy:

Keep things in order. Don't let things collect in piles. Keep dishes put away in an orderly fashion. Keep all the cords for all the appliances bound up with twisties. Collect empty tip boxes from the cell and virus rooms and store in the closet by the door (until they can be filled). Watch that the drugs and chemicals are kept ordered and not left around the lab.

Heavy Cleaning:

About every other Friday, sweep the floors of the main room as well as the cell and virus rooms, and follow the sweeping with a good mopping. Mop with about a 10% solution of bleach in water. Also, remove and replace the lab mat (the white and the orange covering of the lab benches in the main room and in the virus room). Before replacing it, wipe off the counter with ethanol and allow it to dry.

CO2 tanks:

Check the meters on the CO2 tanks in the main room and in the cell room. If the gauges are reading low, then unhook the current CO2 tank and attach a new, full one. When checking the gauges, you'll want to tap them, because occasionally the pointers get stuck and don't give accurate readings. When attaching a new CO2 tank, use the pink tape, placed in the grooves, which will help to create a tighter seal.

Dishes:

Clean the dishes left in the dish basin as often as possible, preferably at least every other day. Clean using the soap solution, and using a sponge or a wire brush. If cleaning glass items, always run the dishes under distilled water after washing with soap and normal water. Always wear goggles and a lab coat while washing the dishes. Place the dishes on the left (generally for bigger items) or the right side of the sink to dry. Once they are dry, put the dishes away in cupboard on southeast corner. Also, remember to change the soapy water in the dish basin about once a month or a needed. Use the concentrated soap under the sink, and add it to clean, distilled water at a ratio of about 1:20.

Incubators:

Check the incubators regularly (maybe weekly) to ascertain whether the pans are filled with water. If not, add distilled water until full. Also, the incubators need to be cleaned occasionally, however it is usually to done after there is a spill and/or mold has been found to be growing therein. (This happens enough that it is *usually* sufficient to clean when these situations arise.)

Inventories:

Virus Inventory:

It is your responsibility to keep the inventory updated, preferably at least once a month. From the inventory sheets (made when a new virus pool is created), extract the pertinent information (such as virus name, virus #, cell type, date, where kept, color of lid, and how many vials there are) and add it to the permanent virus inventory list. (As for now, this list is kept in room 127 on the computer, and must be completed and printed out there. But that is likely changing soon, as everything is slowly evolving into a master computerized network…)

Cell Inventory:

It is also incumbent upon you to keep the cell inventory updated. This will be a lot less of a chore, for there are fewer cells frozen down than viruses frozen down. The list is also on the computer in room 127. The information needed will be on the list made when the cells are first frozen down.

Neutral Red

Staining

When Dr. Barnard places the sign on the incubator that tells you to do neutral red that day, here's what to do. Take the plates to be stained into the virus room. Wearing gloves, goggles and a lab coat, add 100 uL of the neutral red solution to each well of the plate. Cover the plates in tin foil and return them to the 37 degree Celsius incubator. (After around two hours (enough time for the living cells to take up the dye), bring the plates back to the virus room and, in the dark, suck off the fluid from each plate. Rinse each plate twice with 200 uL of PBS in each well. After the second rinse, and the plates are thus dry, recover them with the tin foil and store them in the back of the virus room.

Extracting and Reading:

This is done as often as possible, but the month's work must definitely be done by last week of month for Dr. Barnard's monthly reports. Take the plates that are being stored in the back of the virus room. Add 200 uL to well of each plate (in the dark). Cover the plates with tin foil again and place them on the Vortex Genie in the main room, gently shaking them for 30 minutes. After the time has passed, bring the plates upstairs to the Microplate Reader. Set it according to the directions given you by your supervisor (generally using the 540 nm and 405 nm filters). Wipe off the bottom of each plate with 70% EtOH and dry it before placing it in the machine. Save the data on the computer and write it on the

printout from the printer. Bring the plates back to the lab to be autoclaved, and give the printouts to your supervisor or to Dr. Barnard.

Recycling

When there are empty boxes (from the mail, from flasks, plates, etc), you will have to minimize them and fold them and bring them to the recycling bin behind the VSB.

Solution Preparation: (see Lab Procedures Manual for further information)

DDH2O:

When the double-distilled water level is low in the 25L container, refill it (with at least 10L) from the distiller upstairs in the washroom.

Ethanol:

Your job includes keeping tabs on the stock of ethanol (EtOH). The EtOH is kept in the flame-resistant cabinet under the south counter. If the amount to gallon containers is low, go to Chem. Stores and, with an "A number" from your supervisor, buy a couple more gallons of ethanol (100%, 200 proof). There is an EtOH bottle kept specifically for 70% EtOH. Keep it full of 70% EtOH (by mixing 100% EtOH and water in the right ratio). Furthermore, keep the spray bottles (in the main room, cell room and virus room) full of 70% EtOH.

Neutral Red:

Your job includes making sure there are one to two bottles of neutral red for use in the virus room at all times. It is made in a flask covered in tin foil (because N.R. is less effective if is in the light). In each flask, put 0.17 g of neutral red powder and 4.25 g of NaCl and add 500 mL of DDH2O. Stir for at least half an hour. Make sure that there is tin foil over the opening placed on semi-tightly. Then, autoclave the solution on the liquid cycle for 30 minutes. Return the flasks to the virus room for further use.

PBS (Phosphate Buffered Saline):

There is a big, 25 L container for non-sterile PBS. When it gets low, in it put 85 g NaCl, 5.6 g Na2HPO4, and 1.4 g KH2PO4. Add 10 L of DDH2O and mix until in solution. (Occasionally you will need to make fresh, sterile PBS for experiments. Use 8.5 g NaCl, 0.56 g Na2HPO4, and 0.14 g KH2PO4 in 1000 mL. Sterilize the solution by autoclaving it, in two 500 mL containers, with lids loosened, for 30 minutes on the liquid cycle.)

Secondary Sodium Citrate:

This is made by mixing 21.01 g of citric acid (C6H8O7-H2O) into 200.00 mL of 1N sodium Hydroxide. Q.S. DDH2O until you have 1000 mL. Filter in the cell hood and store.

Sorensen Citrate Buffer:

For use in neutral red extraction, this buffer is made by adding 121.6 mL of 0.1 M Secondary Sodium Citrate and 78.4 mL of 0.1 M of HCl to 200 mL of 100% ethanol. This is kept in the virus room.

Other Solutions:

Keep a good stock of 0.1 M HCl and 1.0 M NaOH in the cupboard on the south side of the lab. Also, be prepared to help your supervisor with other solutions at various times.

Supplies:

Keep these supplies on hand at all times: gloves, pipettes, autoclave bags, flasks, and plates (all found in the storage room upstairs).

Tips:

As for the empty tip containers, you will need to replenish them with tips. The tips come in clear bags, kept in the closet by the door. Take these and use them to fill the empty tip containers. When filled, seal the container off with a piece of

autoclave-signaling tape. Place the containers in the tray and autoclave them for fifteen minutes on the fast cycle. Remove and stack in the closet by the door with the rest of the tip containers. Be sure to not let the supply of tips dwindle too low. (If you see that there are less than about 20 containers, you will want to fill more containers as soon as possible, so as to always have tips available for use.)

Virus Pools:

Making virus pools:

First of all, find out the information needed for the logbook, which is: virus using for the pool and its number, the concentration at which you'll be using the virus in the pool, the new virus number, the cells in the flask you'll be using, the confluency of the cells, and the date. In the logbook also write a brief procedure (based on previous entries). On the cell flasks, write the virus name, the new number, the date, and the confluency (in the bottom right corner). Make sure that there is always one flask per cell type for a cell control.

Once those preliminary steps are taken, get the virus out of the freezer (marking it in the permanent virus inventory list as well as the inventory book by the freezer) and begin thawing it in the water bath in the virus room. Put MEM 0% in the test tubes, one test tube for each flask. (If the flasks are T25, use 1mL MEM, if T75, use 2mL, and if T150, use 4mL.) Add virus to the appropriate test tube (never to the cell control) at the right dilution. (1:50 is standard. For example, if you have a T75 flask, you will have 2 mL of MEM 0% in it. Dividing that by 50, you obtain 40 uL. Thus, you use 40 uL of virus into the 2 mL of MEM in the test tube. At 1:50, in a T25, use 20 uL, in a T75, use 40 uL, and in a T150, use 80 uL of virus.) (If it is an RV pool, also add MgCl2 from the fridge at a ratio of 1:100 in addition to the virus.) Aspirate the growth media

from the flasks and add the media from the test tubes to the appropriate flask. Incubate the flasks for one to two hours in the incubators (37 degrees for all but RV, which goes in the 33 degree incubator.) Then add MEM 2% to each flask (5 mL for T25, 10 mL for T75, and 20 mL for T150), and return them to the incubator, where Dr. Barnard will watch for them to be ready to be frozen down (which is when most of the cells are infected and it's most potent).

Freezing down virus pools

When Dr. Barnard approves freezing down the virus, you must first label cryovials. For each T25 flask, label 7 vials. For a T75 flask, label 13 vials. For a T150 flask, label 26 vials. Generally, viruses are stored in 1 mL increments in 1.2 mL vials. (Exceptions: RSV is stored in 2 mL increments in 2.5 mL vials. Ad, RV, PIV, PT, and PCV are generally stored in 1 mL vials in 0.5 mL increments, because they are usually more potent. Ask your supervisor for specifics.) Label the vials with the virus name, the virus #, and the date. On the lid write the virus number. Bring the vials to the virus hood and loosen the lids. Use a cell scraper to scrape the sides of the flasks up and down, to loosen the cells. With a 10 mL pipette (or a 5 mL pipette if you are measuring increments of 0.5 mL), mix the solution and wash it down the sides a few times. Suck up 10 mL and put it quickly in the vials in the correct increments. In one tube, you will only need to put in about 0.3 – 0.5 mL, which will be used later in a virus titration. Discard the empty flasks and the cell control flask, and tighten the lids on the vials. Find a box in the freezer with room in it and put the vials there. Mark on the virus stock sheet (from which the virus inventory will be updated) the virus name, virus number, cell type, date, where kept, color of lid, and how many vials there are.

Vacuums:

Check to see that the vacuums in the cell and virus rooms are turned to the off position before you leave for the day. (The vacuums are expensive to keep going and this will save the department money.)

Water Baths:

Make sure the water baths are turned off as you leave nightly. Also, make sure they have sufficient distilled water in them. If not, fill them. They should be cleaned about once a month.

For any further information or clarification, please refer to the Lab Procedures Manual and/or inquire of your lab supervisor. Best of luck to you. Enjoy!