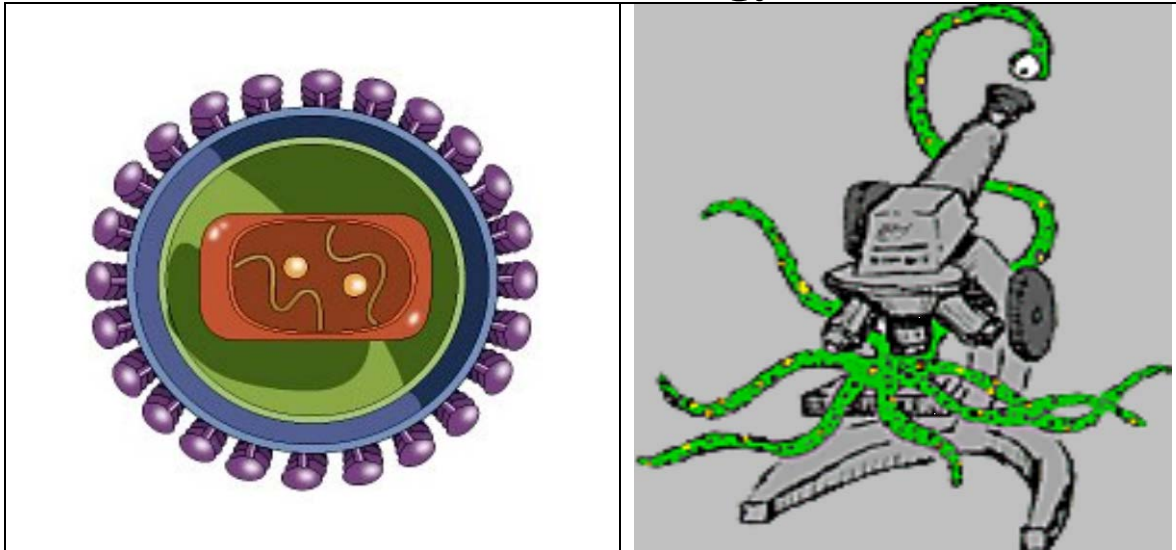


College of the Canyons Biological Introduction to Biotechnology: Custom Lab



MEDIA PREPARATION: Sterile Techniques: Media Transfers, Simple Media Preparation and Autoclaves

Version 8-16-12

- Sterile technique is an informed technique. You need to know exactly what you are doing before you have a chance of doing it in a sterile manner.
- The techniques are a rich mix of theory and practice and require both patience and attention to detail.
- The process can vary in complexity from simple steps taken to minimize contamination (as seen in the kitchen for example) to precise protocols and “bunny suits” seen in disease containment facilities. Whatever the process, you are assessed two ways. First is the familiarity with the theory and practice of the lab and second is the outcome. The sterility of your samples is the most potent proof that sterile technique has been accomplished / observed.
- These lab activities will consist of some sterile transfer work using a variety of different techniques to see if the media is transferred in a sterile manner.
- Media (for use in bacterial culture) will be prepared and sterilized in preparation for pouring Petri dishes, aliquoting liquid media, etc.
- Autoclaves will be operated and the accompanying theory and technique behind the device briefly discussed.
- Solution preparation adjunct (dimensional analysis (DA)) will be reviewed to assist you in making correct calculations and subsequent correct media preparation.

For more information on the College of the Canyons Introduction to Biotechnology contact Jim Wolf, Professor of Biology/Biotechnology at (661)362-3092 or email: jim.wolf@canyons.edu or check out our web site at www.canyons.edu/users/wolfj

Objectives:

- Complete a series of sterile media transfers in a sterile field created on the lab bench (using a “range” of volumetric devices).
- Use calculations to determine the amount of reagent required for simple and complex media preparation.
- Review additional techniques for solution concentration calculations (for complex media) as needed using dimension analysis adjunct.
- Review and implement the key steps in the operation of an autoclave for both liquid and dry media sterilization.
- Pour Petri and contact plates using sterile field technique previously described.
- Review videos on sterile techniques and use provided notes to highlight key aspects.

APPLICATION NOTE: The lab will span two labs, occurring over sequential lab periods. Please read the entire lab on the first day. Ideally, you should read the entire lab before starting any lab, but lets be realistic. Last minute protocol reviews, while not ideal, do happen. So make up for any lapses in responsibility by reading the lab fully. Understand what you are responsible for making today, what will be looked at and prepared in the following lab, etc. Towards the end of the first day of lab is a list of *most* of the items you will be preparing. Be sure to CLEARLY label everything. Also, remember you will be working in a group of 4. Some aspects of the lab will need to be completed by each student, other aspects you will need to complete in pairs, and still others will be the responsibility of the group as a whole. Suffice to say, there are a lot of details and make sure you and everyone in your group completely understand all of the significant elements. As always, feel free to ask the professor if something needs clarification.

Lastly, this is a technique based lab that should not appear in your lab notebook (WAHOO!). Your “grade” will be decided by the quality of the media you prepare! Sterile, clearly labeled, completed sets of material get a passing grade. Contamination, confusion, obvious flaws (poor labeling, etc.)...well you will want to work as hard as you can to avoid this.

Background:

All the lecturing in the world will not validate your effectiveness in sterile technique (and related media preparation, cell culturing). There are wonderful farmers who have never cracked a textbook on plant culturing and many academicians with “black thumbs” (meaning they can not grow anything). The fusion of the academic and the applied is best seen in sterile technique. While there are many points, here are a few things to think about:

- Know the procedure *well before you begin.* Your technique will suffer if you need to get up from the lab bench, check equipment, paperwork, etc. So read ahead, read completely, make notes (perhaps post detailed ideas next to place where the work will be completed).
- Work quickly and accurately. The longer and more laborious the process, the greater the likelihood of contamination.

- We will also review some resources (videos, lecture, etc.) to help familiarize you with some of the key ideas and assess your technique in the following lab by looking for contamination in the samples you have prepared.
- Lastly, practice, practice, practice.

To make solutions of media, simple calculations are needed. As the media becomes more complex, or the chemicals more exotic, the calculations will become more exacting. We looked at these more involved solutions in previous labs (see metric system and dimensional analysis lab). After reviewing the needed SOPs (standard operating procedures), the needed supplies will be gathered and a sterile field created. Working left to right (to allow for one to follow what is contaminated and what is not), a set of 1.5 ml microtubes of Luria Broth (LB) will be aliquoted into smaller containers. This allows for researchers to grab a small aliquot as needed and lessen the risk of contaminating the stock solution. You will try transferring the solutions using three common volumetric devices: serological, transfer and micropipettes. These solutions will be assessed for sterility in the following lab. You will also prepare some simple media for culture plates. After the amount of dry media is determined, 100-300 ml of liquid media will be made and autoclaved according to a standard operating procedure (SOP). The prepared Petri dishes and liquid cultures will be used in later labs to assess bacterial contamination as well as possibly provide needed reagents for misc. labs (i.e. cleanroom course, microbiology, etc.). The sequence and scale of lab activities may be altered for time management purposes (so keep your ears and eyes open).

DAY ONE ACTIVITIES: BACKGROUND READING

Media Preparation:

Calculating the amount of media required.

Commercial manufactures of media make available standard recipes for their preparation. The provided information often comes in grams of dry media required for a liter of liquid media. Rarely is exactly one liter needed, so simply multiply this amount by the number of liters needed (multiple liters or fractions of a liter). Weigh out this amount of media and add liquid to attain the final total volume. This technique is called “bringing to volume” (BTV) and is NOT adding the media to the final needed volume as this will result in inaccurate volumes as well as too much being made. For instance, if a media requires 40 grams per liter, adding 40 grams to a liter of liquid will result in more than a liter being created. So, add 40 grams to a container and bring the total volume to one liter. To verify that you understand this, calculate and describe how you would create:

- 2 liters of 40 grams/liter media:
- 1300 mls of 65 grams/liter media
- 750 mls of 35 grams/liter media

Double check your answers by checking the lab key.

Preparing the Media:

On the last page of this handout is a set of “Media Request Forms”. They are derived from forms used in biotech companies (i.e. Amgen and Baxter, etc.). At the end of lab, you should complete a number of these forms (more available upon request) and attach one form to each of the items you need autoclaved. Be sure to review your form with the professor (BEFORE making the media), and make every effort to ensure that these forms are correct and the media prepared in appropriate for the assigned lab activity. Add the dry media to a container that is approximately twice the needed final volume (this is to help prevent the media from boiling over when autoclaved). So... for example, make 200 mls of media in a 500 ml flask, 500 mls in a one liter flask, etc.). When in doubt, use a larger flask... Mix the media using either: simple agitation, a stir bar (with or without heat). The specific process varies with the type of media. There are just a few variations on the basic technique. Place the lid **loosely** on the bottle. Secure with the lid with a piece of autoclave tape. Just a small piece is needed. Again, do not seal the container (**it will explode in the autoclave**).

Label container with: media, recipe, date, your initials and any other information as noted necessary.

Prepare media as directed in lab. You will be making at least 3 media per “team”, so be sure to understand all steps. As you will be pouring culture plates and Petri dishes consider the type of media, plate size and number. Small (65 mm) plates require about 10 mls per plate and large (100 mm) require about 20-25 mls per plate. Some times plates are poured so that the media is thicker (and requires more liquid be added to the plate) especially when plates need to grow organisms for a long period of time or at a higher temperature. What ever the volume needed, always add about 10-15 % (to the total volume of the media prepared) more to accommodate for spillage, evaporation, mistakes made while pouring, etc.

DAY 2: BACKGROUND READING

Complex Media Preparation:

Calculating the amount of media required.

The “stock” media has been prepared and autoclaved. Stock would be the “LB” in a “LB”/AMP, or “LB”/AMP/ARA. It is essentially the “stock” you start with when making a more complex soup. You will calculate the amount of new reagents needed to bring the stock solution into the needed concentration of new complex media. You will want to review and use the techniques learned in last lab, dimensional analysis and metric labs and really plan ahead. For instance, as the temperature gets cooler as time goes by, you need to be prepared to add the new chemicals as needed and ensure both the right amount and order are observed. Some chemicals can simply be added to the stock media prior to autoclaving or when the sample is very hot (like ethidium bromide, which is added to DNA gels to help stain the DNA). Other substances are temperature sensitive. Many antibiotics need to be added only after the solution has reached the correct, cooler temperature (65 °C is common). Any cooler and the sample may not be liquid long enough to pour all plates (before the agarose sets up (solidify). This is tricky and requires planning, calculations, and a good sense of timing. For instance, consider a set of plates (100 mm) that are of one of three types:

Luria Broth agar (LB), LB with ampicillin (amp), or LB/Amp/Arabinose (LB/Amp/Ara). This set of plates is commonly used in genetic engineering experiments where the

antibiotic is used to keep out unwanted bacteria and the arabinose acts as a switch turning on certain genes (recall the 107 pGLO labs). Now, assume you need 5 of each plate...how would you do this?

First, figure out how much LB agar is needed. 15 plates each require 20 mls (minimum) of media and that all of the plates need the LB media in them. You will want some extra to allow for inaccuracies in pipetting, water loss, accidents, etc.. so prepare 350 mls. Autoclave and cool to 65 °C (as outlined previously). Pour 5 plates of LB. If you do not know what to do next, return media to the water bath as you do not want it setting up (turning solid) while you wait. Now add the ampicillin. Why? This will make the media LB/Amp. As there is no LB/Amp available as a media, so this is the next step is adding ampicillin, but how much ampicillin? Well, this is where things get a bit technical. Ampicillin is often stored in a liquid form, frozen for ease of use and alleged longer shelf life. 100 mg/ml is the stock concentration (in previous labs we have reviewed how to make this type of solution using dimensional analysis). In the final media, the amp needs a concentration of 0.1mg/ml to be lethal to the bacteria. To figure out how much to add, use a $C_1V_1=C_2V_2$ relationship. C and V stand for concentration and volume respectively. So set up the proportion as noted:

$$100 \text{ mg/ml (V1)} = 0.1\text{mg/ml (250 mls)}$$

Where did these numbers come from?

The 100 mg is the concentration of the stock ampicillin. V1 is what we want to know (how much of this stock should we add?). C2 is the final concentration (the lethal limit, see above) and 250 mls is the amount of LB left after we poured 5 plates (350 mls – (5 x 20 mls/plate)).

Solving for V1 =0.25 mls or 250 ul. I always tell students to do the math twice and to ask your self does it make sense? The difference between the two concentrations is 1000 (move decimal 3 times). Since we need ¼ of a liter (250 mls), we need ¼ of the total volume of 1 ml or 250 uls...so it makes sense! Add the ampicillin to the media using a micropipette (see previous practice exercises to refine technique as needed).

Here are a few other technical notes:

- The media needs to be no warmer than 70 °C. Any warmer and the ampicillin may be destroyed (recall it is kept frozen or very cold prior to use).
- Consider adding a bit extra. 250 ul is the calculated amount, but ampicillin is a lot like produce (fruits and vegetables) in that it has a shelf-life. So by adding extra, you can overcome this problem. I routinely add twice the amount as this ampicillin is inexpensive, and the extra does not hurt the outcome (so for our protocol, consider adding 500 ul). If the ampicillin is not strong enough, the science gets really messed up!
- Work quickly as the cooler media is now getting closer to “setting up” (becoming solid).
- Remember to keep things straight. The LB plates look just like the LB/Amp plates, so keep it straight.
- Lastly, save the plastic sleeve that the plates came in. They are ideal for storing plates in the refrigerator.

So try this one, how much arabinose should you add if the stock solution is 100 mg/ml and the final needed concentration is 5 mg/ml? Show your work and ask for verification (work with colleagues or ask the professor).

Your group will be assigned at least two complex media pours according to what items are needed for future labs, ordered by other labs, etc. The assignment will be very specific so keep good records and be prepared to make at least 2 and as many as 4 complex media and accompanying pours over coming lab periods.

Plate storage. Always label plates on the bottom of the plate (the bottom follow the media, not the lid!) After 15-20 minutes (the agar has set up), use a lab marker to ID each plate. Abbreviations are fine if they are clearly all agreed upon. If a lab routinely uses only a few types of plates, an even quicker “stripe technique” can be used. Place the plates in a small stack. Make sure the tops are on top, bottoms on bottom and take a permanent marker and run a strip down the length of the stack of plates. Hold the plates firmly, but gently. As the marker is drawn down the side of the plates, it will “skip” some. Be prepared for this, as knocking the plates over For the 3 plates used in the Amgen lab, LB/ would be black, LB/AMP would be a black and red stripe. The LB/AMP/ARA would have stripes of black, red and blue). However you label the plates, do not forget the date and initials. Lastly, you will stack the plates and carefully put the sleeves back onto the plates (the sleeve is the plastic the Petri dishes come in, it should be saved and used at this time to ensure the plates last a long time in storage). Tape shut and label the sleeve with plate type, date and your initials.

DAY ONE ACTIVITIES: MEDIA PREPARATION

Required Media from each TEAM. The team will be four students. The team will need all of the plates, media, etc. as noted. Review this list carefully. Consider how much of each media you will need, appropriate vials, sizes, labeling, etc. By then end of the first day’s lab, you team will be expected to provide the professor with all of the agarose vials, sterile water vial, Q-tips and any last minute noted samples that need to be autoclaved in preparation for the next lab.

Media Needed: NOTE: to ensure you will have enough media, make sure you prepare 25 mls for each 100 mm plate (20 mls may work, but given the small sample sizes and novice nature of the technicians (no offense), a little extra media is a step in the right direction.

<p>Amgen Media: 2 sets per team of four: 2 100 mm LB plates 2, 100 mm LB/AMP plates 2, 100 mm LB/AMP/ARA plates</p>	<p>Cell Culture Media: 2 sets per team of four: 24, 65 mm TSA plates, 12, 65 mm SDA plates 12, 65 mm TSA with Tween (contact plates).</p>	<p>Extra: 30, 1 ml LB aliquotes (each student prepares) 8, 1 ml sterile water, 1.5 ml microfuge tubes (each student) 6, test-tubes with 4 Q-tips (each student, misc. additional sterile media (microfuge tubes, etc) as noted</p>
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Autoclave Operation:

Note: Different autoclaves have specific operational steps. The following are specific, to College of the Canyons' autoclaves. When in doubt, ask someone who is familiar with the institution's autoclaves and their specific functionality / operation. This said, most of the following ideas apply to all autoclave with respect to general process, safety, etc.

- Complete autoclave operational log as needed (note, our autoclaves do not have operator logs, but most industry ones do). The log would include: operator I.D., run time, dry or wet run, date and other salient comments.
- Remove any items left in autoclave. **NOTE: Do not open the device unless the pressure gauge reads 0 (zero) and the temperature is under 180. Even with these readings, steam may still come out, so open cautiously. Use heat resistant gloves as needed to handle the media.**
- Make sure the drain is closed by turning in noted direction. Be very precise, as small leaks associated with inaccurate valve placement will cause the autoclave to leak. This leak will stop the autoclave from reaching lethal temperatures, thus not work correctly.
- Using tap water, fill the reservoir to the fill line noted on the bottom (or interior side) of the device. These devices require about 6 liters of water FYI. Never use deionized or distilled water unless expressly told to do so.
- Place all the items to be autoclaved into a tray and place into autoclave.
- Set the exhaust switch to either "slow for liquids" or "fast for dry items". Specific verbiage varies, but slow is used for liquids, media, plates (which turn to liquid upon heating). Fast is for flasks, scalpels, hardware, etc. Essentially, anything that can boil gets a slow cycle.
- Close the door carefully, making sure to not catch any items in the door and that the seal is secure and solid around the door's entire periphery.
- Turn dial to the time required. Most media requires about 30 minutes. Longer times may be required for the decontamination of Petri dishes, virulent organisms, larger loads, etc.

General Pointers: Background

To check if the autoclave is really working involves sterilizing spores of a specific strain of bacteria. Certain companies provide sealed, glass containers with a small amount of liquid media and a few bacterial spores floating in this "vital media". The vial is placed in the autoclave and cycled. The vials are then checked periodically over the next few days. If the autoclave is working, the vials remain unchanged as the bacterial spores are dead. If the autoclave is not functioning correctly, the spores will "hatch" and the bacterial will start to grow in the vial. The "vital media" is designed to detect metabolism (or life, hence the word vital). As the bacteria grow, their waste products shift the pH, etc. and the dye changes color. So, a color change in the vial indicates a autoclave that needs to be calibrated, fixed, etc..

The autoclave will take about twice as long as the time set to complete a cycle. So, if you set the cycle time for 30 minutes, expect it to take about one hour. This delay s due to the time required to heat, decompress, etc. Some autoclaves are hooked to steam systems or have other mechanism that can cut this delay time down substantially. This is because time is required to get up to temperature and pressure as well as cool down and

decompress. Once the cycle is complete; again check pressure and temperature. Open slowly and be aware of steam. Use gloves and remove items as directed.

For most media, the vials should be placed into a water bath which is set @ 65 °C. Depending on the size of the vial, it will take 15-30 minutes to cool. This is required for safety of handling as well as the high temperature which can cause excessive condensation in the containers, damage future chemicals to be added (especially antibiotics), and is all around just easier to handle once the media has cooled some.

LASLTY: When containers of media are placed into water baths, be aware of the effect of displacement. Each container sits in the water bath, with enough water around it to allow for adequate heating. If you add or remove a container, the water level changes some. This may cause bottles to float, move, etc. Be aware of the consequence of removing or adding additional sterile media bottles from the water bath. The floating bottles may flip and sink, take in water, etc. This would obviously contaminate that media, water bath, etc! Also, watch out for the water level getting too low. If there is not enough water around the bottle, the media may begin to cool. Add only hot, de-ionized water to the water bath. ***In a nutshell, watch the water bath closely and be aware of the consequences of removing or adding vials of media!***

Luria Broth Transfers:

Watch the following video on Basic Sterile Technique. A set of notes for the video is on the last page of this lab FYI. In a few weeks, you will be working in the tissue culture hood and previewing some additional videos (and this one again). Remember to read the entire procedure before starting as you will need to gather essential items and be comfortable with the key aspects of the protocol. In the future weeks, you will have plenty of opportunities to review the technique. So the following points on sterile technique are only broadly outlined to encourage you to jump in and to not freak out. Remember, sterile technique is a fusion of applied and theory, so proceed with caution, but be confident in the fact that you will be allowed to repeat and refine your technique. With your new-found fear of bacteria; prepare a sterile field in the manner outlined below: spray the surface with alcohol and let stand for 2 minutes. Dry surface with clean towel and put on gloves. Place needed items onto left side of the “field”, using alcohol spray to sterilize individual items as demonstrated. Take care with media and cultures to ensure alcohol does not kill or damage the sample. See below for needed items and repeat sterile field prep for each of the transfers.

Again...prior to starting the following two activities (Luria Broth transfers and media preparation) review the process and walk through the process using non-sterile items. The effectiveness of the process is directly related to your familiarity with the steps and equipment. Remember, sterile technique equals informed technique!

LB Transfer: Keep each type of transfer separate. You will ultimately place all 30 LB samples in a common rack, and will want to be able to tell them apart for the later analysis on day 2. Foil wrapped sets of 10, 1.5 ml microfuge tubes have been prepared and autoclaved for you (note autoclave tape). You will want to get three packets and inspect briefly for tears, etc. If in doubt, grab another packet. Also grab a sharp tip, lab line marker. Write legibly on the cap of the microfuge tube. You will need 6 letters (ex

LB,MP,JW) to ID each tube, so write clearly, neatly, etc. You will also need 3 vials of Luria Broth (11 ml in a 15 ml falcon tube (blue lid, pointed bottom and ml demarcations.)

Using a **1 ml micropipette**, tips and sterile 1.5 ml microfuge tubes, transfer 1 ml of Luria Broth to 10 separate microfuge tubes. A new tip is only needed if the pipette tip comes in contact with surfaces or contamination is suspected. Keep each pile of 10 separate and clearly label as needed. So label the microfuge tube lid "**LB/MP**" using a sharp point, lab-line marker.

Repeat process with a sterile **transfer pipette**. Replace pipette if tip comes in contact with surfaces or contamination is suspected. So label microfuge tube lid "**LB/TP**" using a sharp point, lab-line marker.

Repeat process with a 1 ml **serological pipette**. Replace pipette if tip comes in contact with surfaces or contamination is suspected. So label microfuge tube lid "**LB/SP**" using a sharp point, lab-line marker.

At the end of the process there should be three piles of ten, 1.5 ml microfuge tubes each with about 1 ml of Luria Broth in them. Label all of the tubes clearly with your initials and double check method of treatment and place in appropriately labeled rack at the front of the class.

DAY TWO ACTIVITIES: ASSESSMENT

Luria Broth Transfers: Assessment

From last lab you should have 30 labeled vials of Luria Broth (LB). They have been at 37 °C to *encourage growth* of bacteria or other contaminants. To assess them you will do a quick visual inspection for obvious contamination. While there are many methods to check for contamination, the emphasis is on the practical. If you open the vial to do more sophisticated tests, you risk contaminating the very vials you hope are sterile! So, an additional non-contaminating test is to spin the sample (centrifuge) and look for a pellet of bacteria or other contaminant. **When in doubt, throw it out!**

Luria Broth Transfer Assay:

Create a positive and negative LB sample by transferring 1 ml of LB broth with bacteria contaminate (positive control) to labeled microfuge tube and 1 ml of clean LB labeled (negative control tube). Both control samples are available from the professor. Remove a set of ten identical microfuge tubes from the respective place in the rack (i.e. serological, micropipette, etc.) and place in centrifuge with positive and negative controls. Make sure tubes are balanced in the centrifuge by placing samples of equal volume on opposite sides of the rotor (ask if unsure). Space permitting, put in additional tubes from other samples or other students. Some of the larger rotors hold more than 60 samples, so be sure to use a full rotor any time you do a spin. Be sure to note what tubes are where (in the rotor) if the labeling of the tubes is not clear, and keep like samples together for easier diagnosis, handling etc. Spin at maximum RPM (14,000) for three minutes. Gently remove samples and try not to agitate. Note the presence of a pellet on the bottom of the positive control tube. This indicates contamination. ANY discoloration, irregularity, etc. is basis for discarding the sample. Always err on the side of caution. Using the controls for

comparison identify the number of contaminated tubes per the three assays as noted on the following page.

Assay Results from Luria broth sterile transfer:

- Transfer pipette protocol: number contaminated_____ number sterile_____
- Serological pipette protocol: number contaminated_____ number sterile_____
- Micro-pipette protocol: number contaminated_____ number sterile_____

Dispose of ANY contaminated samples in a biohazard container. Save the good samples and using the micro-dot labels (available from the instructor), label the top of tube with the letters LB and place in a labeled rack at the front of the class.

DAY TWO ACTIVITIES: MEDIA PREPARATION.

Simple and Complex Media Pours:

Get all items needed, sterilize items with alcohol and create a sterile field as noted previously. Place a set assigned numbers of Petri dishes. The number is function of comfort, ease of pouring as well as how many are needed. Autoclaved media may be taken directly, allowed to cool slightly and poured. If needed, a water bath set @ 65 °C is used as this is warm enough to allow for some time to pour prior to media setting up (solidifying), but cool enough to minimize condensation in the Petri dishes and/or allow media like antibiotics to not be damaged by excessive heat. Here is a neat trick. Using a thermometer to check media temperature may contaminate the media, so you can grab the flask (cautiously!). If it is cool enough to hold in you hand, it is about 60-70 °C. If too hot to hold, it is above 70 °C. While not bulletproof, this procedure is often accurate enough for most procedures! **Regarding making complex media**. You will need to add the correct amount of ampicillin and then arabinose (based on earlier calculations). This amount should be added in sterile manner (transfer or micropipette are best choice). Swirl to mix and recall that the addition of the amp or ara means you can no longer reheat the LB agar. This will mean you need to work quickly, and if you need to keep the vial warm for a while, replace the vial into the water bath. This brings up another point. Water baths will keep samples warm, but make sure the sample is stable in the bath. Too much water in the bath or not enough media in the vial could result in the vial slipping under the water in the bath and really contaminating the sample...So, think ahead when taking samples in or taking samples out of the water bath. Be careful to avoid getting your samples contaminated or to risk the contamination of your colleague's samples!

Using serological pipettes to transfer 10 mls of media (for 65 mm plates) or 25 mls for 100 mm plates (10 and 25 ml serological pipettes and pumps are available). Open plate partially and add media. Swirl slightly if needed to spread out the media and place lid slightly askew.

Repeat with reaming plates. Try not to work over poured plates, replace pipette as needed and work quickly.

Plate will set in about 10 minutes. Once media looks more cloudy (this visually verifies that the media has solidified), close lid completely.

Flip over and identify plate on the BOTTOM with date, media type. There a lot of tricks to move this along and if you're curious, feel free to ask the professor for a few or revisit the lab notes to examine the "stripe method" of plate I.D.

VIDEO NOTES:

Basic Sterile Cell Culture

Work Area: Cell culture cabinet. Do not use to store items, and position reagents so that they do not block vents or laminar flow around individual reagents.

Supplies are nearby, so that person doing culturing does not need to move. Lighting kept to minimum as to avoid free radical oxidation of reagents (unless sterilizing with UV prior to passing cells.)

Passing suspension cells: Sterilizing done with Et-OH and iodine solution.
Scrubbed with aseptic foam onto glove (optional)

Sterile techniques: Sources of contamination: Technician, water bath, excessive supplies in cabinet, length of time of procedure.

Passing Cells: Cloudy sample implies need to transfer.

Note technician's pipetting technique.

All reagents and supplies are in line, care is taken to avoid crossing over, and cap is closely monitored.

Pipette don'ts: do not touch shaft of pipette, open only in hood, close when not in use, and use cotton plugs when possible.

Cell Culture Plastic: Quite variable: FYI...Culture wells, culture bottles and culture flasks.

Equipment: Keep sterile, especially water baths, cell culture hood and incubators.

Microbes Types: Virus, mycoplasma, bacteria (first three, not easily visible, but can often see effects.) Fungal and molds often grossly visible.

Brownian movement: Random oscillation due to molecular bombardment. Can make inert particles move and thus appear living.

Chief sources of microbial contamination: poor sterilization, room air, dust, aerosols (from talking and sneezing), malfunctioning hood, chalk dust.

Final NOTE: While this was mentioned earlier, it warrants reiterating. This lab is a technique intensive lab and should be retained as reference. This lab will not appear in your lab notebook. There will be no quizzes on this material, etc. We will be using some of the media (especially the plates) in labs coming up in the next few weeks. We will be preparing LB, LB/AMP and LB/AMP/ARA plates for the Amgen labs and LB, SDA and TSA with Tween plates (and some extra LB aliquots for testing your technique) for the cell culture lab. While you might only prepare a few of these plates, it is important that you review all of the protocols to ensure you understand how these plates are made, used, etc. It is possible, you may be preparing more plates if the first set of plates, media is heavily contaminated. So, in a nutshell, prepare some of the media, but understand everything!

Lab Skills: Production Request Form

Ordered by:

Solutions Ordered:

Date of Request:

Amount:

Date Expected Use:

Aliquoting Instructions:

Labeling Instructions:

Container Preferred:

pH Correction?

Heating Instructions?

Filtering Needed? Yes/No Autoclaving Required? Yes/No

Storage

Instructions: _____

Reagent(s): _____

Formula Weight: _____

Hydration: _____

Recipe Used(math):

-If molar, begin with 1M solution in grams per liter, show math to product.

-If % solution, begin with 100 mL of 1% solution, show math to product.

Lab Skills: Production Request Form

Ordered by:

Solutions Ordered:

Date of Request:

Amount:

Date Expected Use:

Aliquoting Instructions:

Labeling Instructions:

Container Preferred:

pH Correction?

Heating Instructions?

Filtering Needed? Yes/No Autoclaving Required? Yes/No

Storage

Instructions: _____

Reagent(s): _____

Formula Weight: _____

Hydration: _____

Recipe Used(math):

-If molar, begin with 1M solution in grams per liter, show math to product.

-If % solution, begin with 100 mL of 1% solution, show math to product.

Lab Skills: Production Request Form

Ordered by:

Solutions Ordered:

Date of Request:

Amount:

Date Expected Use:

Aliquoting Instructions:

Labeling Instructions:

Container Preferred:

pH Correction?

Heating Instructions?

Filtering Needed? Yes/No Autoclaving Required? Yes/No

Storage

Instructions: _____

Reagent(s): _____

Formula Weight: _____

Hydration: _____

Recipe Used(math):

-If molar, begin with 1M solution in grams per liter, show math to product.

-If % solution, begin with 100 mL of 1% solution, show math to product.

Lab Skills: Production Request Form

Ordered by:

Solutions Ordered:

Date of Request:

Amount:

Date Expected Use:

Aliquoting Instructions:

Labeling Instructions:

Container Preferred:

pH Correction?

Heating Instructions?

Filtering Needed? Yes/No Autoclaving Required? Yes/No

Storage

Instructions: _____

Reagent(s): _____

Formula Weight: _____

Hydration: _____

Recipe Used(math):

-If molar, begin with 1M solution in grams per liter, show math to product.

-If % solution, begin with 100 mL of 1% solution, show math to product.