BTEC 4200 Lab 2. Media preparation and Autoclave Validation

Background & Reference

Dehydrated culture media first became available to microbiologists around 1900 when a commercial vendor, Difco, began to formulate and sell standardized media in dehydrated form to microbiologists. Over the years many other companies also began to offer dehydrated media, including Becton, Dickinson, and Company (BD). BD acquired Difco in the 1990s, but maintains separate manufacture and labeling of its Difco and BD product lines even though many of the products are very similar. The reason for this is because a standard operating procedure calling for Difco medium might not be able to use a BD substitute, or vice versa.


In this lab you will be making up tryptic soy agar broth and tryptic soy agar plates to use in next week’s lab (“Calibrated Growth Curve”). From the online *Difco & BBL Manual* you will need to download two sections:

“Bacto™ Tryptic Soy Broth/Trypticase™ Soy Broth (Soybean-Casein Digest Medium) · Trypticase™ Soy Broth with 6.5% Sodium Chloride · Trypticase™ Soy Broth with 5% Fildes Enrichment · Bacto™ Tryptic Soy Broth without Dextrose”

and

“Tryptic Soy Agar/Trypticase™ Soy Agar (Soybean-Casein Digest Agar).”

You should find information to include in the background section of your lab notebook in these sections, as well as the directions for making up the media.

You will also do an autoclave validation procedure using *Bacillus subtilis* as a biological indicator, as well as chemical autoclave tape. For a reference on the importance of biological indicators and their placement, you can site the following article:

Methods

You will be working in your same groups as last week (two; possibly three in one group). In this lab, you are going to prepare the media you will need for next week’s lab. You will also perform an autocalve validation procedure using a suspension of *Bacillus subtilis* as a biological indicator. Specifically, each group will make up the following:

16 empty culture tubes, with caps
20 tryptic soy agar (TSA) plates
200 ml of tryptic soy broth (TSB) (made up in a 500 ml erlenmeyer flask)

I am going to provide you with a brief protocol for making up TSA & TSB, referring to the *Difco & BBL Manual*. You will need to develop this into a detailed standard operating procedure, using my brief protocol. In developing your SOP, recall the detail in the aerobic plate count SOP from the FDA Microbiological Methods manual (e.g. is your glassware clean?).

*Tryptic Soy Broth:*

1. Follow the directions given in the *Difco & BBL Manual* for Bacto™ Tryptic Soy Broth (or BBL™ Trypticase™ Soy Broth) with the following modifications:
   - The “Directions for Preparation” gives the amount for making up 1 L; you are making up 200 ml, so adjust accordingly.
   - You can omit the second step in the directions “Warm gently until solution is complete.” TSB usually goes into solution quite easily at room temperature.
   - I recommend that your SOP specifically state that you put the powder in the flask first, then put in half the water, swirl to dissolve, then the rest of the water, swirl to completely dissolve and mix. This minimizes the chance that any stray powder will get stuck at the top of your flask.

2. Make up the 200 ml of TSB in a clean 500 ml erlenmeyer flask. When autoclaving media, it is important to use a vessel that is at least twice the size of the volume being autoclaved. This helps to prevent the liquid from becoming superheated and boiling over.

3. Cover the opening of the flask with a double layer of aluminum foil. The foil cap should be crimped tightly enough so it won’t fall off during autoclaving, but not so tight that the pressure can’t escape from the flask.

4. Fresh medium should be stored at least two days at room temperature to ensure against contamination. We routinely store most sterile media at room temperature for time periods up to several weeks.

*Tryptic Soy Agar:*

1. Follow the directions given in the *Difco & BBL Manual* for Bacto™ Tryptic Soy Agar (or BBL™ Trypticase™ Soy Agar) with the following modifications:
   - The “Directions for Preparation” gives the amount for making up 1 L; you are making up 500 ml, so adjust accordingly.
   - You can omit the second step in the directions “Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.”
• You must make sure that all of the TSA powder is thoroughly wetted before you autoclave it to ensure that the TSA is not burned or scorched in the autoclave. Same thing as before: I recommend that your SOP specifically state that you put the powder in the flask first, then put in half the water, swirl to dissolve, then the rest of the water, swirl to completely dissolve and mix. This minimizes the chance that any stray powder will get stuck at the top of your flask. This is especially critical for agar-based medium, because the powder must be wetted for it to dissolve properly when it heats up in the autoclave.

2. Make up the 500 ml of TSB in a clean 1000 ml erlenmeyer flask. When autoclaving media, it is important to use a vessel that is at least twice the size of the volume being autoclaved. This helps to prevent the liquid from becoming superheated and boiling over.

3. Cover the opening of the flask with a double layer of aluminum foil. The foil cap should be crimped tightly enough so it won’t fall off during autoclaving, but not so tight that the pressure can’t escape from the flask.

4. Once the autoclave cycle has finished and the pressure has dropped to zero, carefully remove the flask. Allow it to cool to approximately 60°C, then carefully pour then molten agar into standard-sized petri dishes until they are about half full (about 25 ml).

5. Allow the plates to solidify, then invert and stack them for storage.

6. Fresh plates should be stored for at least two days at room temperature before use to ensure against contaminants. We routinely store most sterile media at room temperature for time periods up to several weeks.

**Empty Test Tubes:**

1. Come on, now.

**Market Forge Autoclave**

1. We will use the Market Forge autoclaves, of which we have two in our autoclave room. The New Hampshire Biotechnology Center at New Hampshire Community Technical College excellent Biotechnology education site that has an SOP for the Market Forge. Here is the URL:

   [http://biotech.nhctc.edu/](http://biotech.nhctc.edu/)

   and the specific link to the autoclave procedure:

   [http://biotech.nhctc.edu/VLab/Autoclave.html](http://biotech.nhctc.edu/VLab/Autoclave.html)

2. I want to say a bit about “slow exhaust” and “fast exhaust.” These terms refer to how rapidly the steam exhaust valve releases the steam pressure after the sterilization cycle is completed. “Fast exhaust” is for autoclave runs when you **only** have empty glassware or dry goods, equipment, etc. **NO media or liquids of any kind.** Basically, under fast exhaust the steam pressure is dumped all at once immediately upon completion of the sterilization cycle, with no cool-down time. Under slow exhaust, the steam pressure is released slowly, to give the media inside time to cool down.
**Autoclave Validation:**

1. During the autoclave runs, heat-sensitive autoclave tape will be placed on flasks and test tube racks as a rapid autoclave validation method.

2. Tubes of *Bacillus subtilis* spores (3 per autoclave) will be used as biological indicators. They should be labeled and placed at the front, middle, and back of the chamber.

3. After the cycle, let the *B. subtilis* tubes cool down to room temperature. Plate 0.1 ml from each tube onto separate labeled TSA plates (you will be provided with TSA plates for this purpose). Incubate the plates for 48 hr at 37°C.