Media Handling & Sterilization

Once the medium is properly prepared, it is time to dispense the media into individual containers. After the Media is dispensed it is then sterilized. This section contains the procedures for dispensing and sterilizing the medium.

1. Place the container of medium next to the dispensing unit. Place a stirrer in the medium and begin agitation.

2. Place a dispensing hose into medium and begin pumping. Pump the solution back into the containter until most of the air has left the hose. Using a graduated cylinder, calibrate the dispenser to deliver the amount required for each chamber. The amount of media per *Star*Pac*® chamber will vary depending on the type of *Star*Pac*® used and the type of plant material to be cultured. Typical amounts range from 10 mL per chamber for a five(5)-chamber *Star*Pac*®, to 20 mL per chamber for a two(2)-chamber *Star*Pac*®.

3. After the dispenser is calibrated to deliver the correct amount, begin dispensing into the *Star*Pacs*®. After the *StarPacs*® are filled, set upright in a tray. *Star*Pacs*® should be handled in units of five or ten.

4. After the medium is dispensed, fold the *Star*Pacs*® in half, in units of five or ten. Place upright in a paper bag of the appropriate size. Six-pound or eight-pound bags are the most commonly used. Fold the top of the paper bag down twice and tape shut. Fold the top toward the seam, not away from it. This will prevent the seam from splitting after autoclaving. Autoclave tape is preferred, though any tape that can withstand autoclave conditions will work. **Do not seal the *Star*Pacs*® prior to autoclaving.** Sealed bags will explode if autoclaved, due to the expansion of the air and liquid inside the *Star*Pac*®.

5. Each paper bag should be labeled with the type of medium. Use a permanent marker so the writing will not fade after autoclaving. Place the paper bags into an autoclavable tray and place in the autoclave.

6. The medium should be sterilized for a minimum of 20 minutes at 121°C and 1.2 kg/sq. cm. (15 psi). Temperatures above 125°C can result in shrinkage or melting of the *Star*Pac*®. Sterilization times greater than 20 minutes may be required for large volumes of media. For large volumes, 25 to 30 minutes may be required for proper sterilization. For proper autoclave use refer to the manufacturer's guidelines.

7. Remove trays from the autoclave after the autoclave has finished the sterilization cycle. Shake trays when they are removed from the autoclave to disperse the gelling agent within the medium. This will promote uniform medium consistency. Store trays in the transfer room or the cleanest room available.

TROUBLESHOOTING

These procedures have been tested and used successfully in a commercial production setting for some time. Nonetheless, problems may still occasionally occur. The following two problems are the more common problems encountered in a production setting. It is by no means all inclusive and there may be better ways to solve the problems mentioned. This serves as a starting point but it is up to the individual laboratory manager to determine the best solution for his/her particular situation.

Media Hardness

A frequently encountered problem is variation in media hardness. Most common is the medium being too soft. The first thing to check is the amount of gelling agent. Next try to determine if the pH is correct. If the pH is too low (less than 4.5) agar will not gel. If Phytagel is used, the conductivity of the media may be too low. The most common reason for inconsistencies within the batch is improper agitation during the dispensing or failure to shake the trays when they are removed from the autoclave. Sometimes the amount of gelling agent used must be adjusted. Agar varies from lot to lot because it is derived from seaweed. If no reason is discovered for the media not setting up correctly (too hard or too soft), adjust the amount of gelling agent used.

Contaminated Media

One of the most severe problems that can occur is contaminated media. Contamination is the growth of an unwanted microorganism (bacteria or fungi) in the culture medium. The danger lies in not detecting it until after the medium is planted with cultures. The most common reason for media contamination is too short a sterilization time. Regardless of what type of autoclave is used, the greater the load the longer the sterilization time must be. Another possibility may be the incorrect temperature and pressure. The temperature must be at least 121°C and the pressure must be at least 1.2 kg/sq. cm. (15 psi). Be careful not to go too high or the media may overcook, or the *Star*Pac*® may shrink or melt. The autoclave should also be checked for proper temperature and pressure calibration. The most common contamination encountered in this situation is bacteria. The most common being *Bacillus sp.* Occasionally contamination can occur after autoclaving if the quality of the bags used for sacking the media are poor. After sterilization the glue holding the seams of the paper together may separate leaving openings for airborne contaminants. Typical types of contaminants encountered are fungi. When contaminated media is discovered, discard the questionable media and prepare more after making proper adjustments. Mark each batch noting what the adjustments were for future reference.