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Growth and Maintenance of Culture

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INTRODUCTION

In molecular biology many microorganisms are involved in the experiment and we need specialized techniques to handle them. Growth media must have essential nutrients for proper growth and maintenance of culture. Autoclaving of the media and discard are directly and indirectly involved in good culture growth. While working with the microbes, issues related to biosafety and biohazard must be attended for personal safety and environmental health.

LEARNING OBJECTIVES

- Maintenance of microbial culture
- Autoclaving procedures
- Biohazard and biosafety

Maintenance of Microbial Culture

Agar Plates for Short-term Storage: Quadrant streak method must be used to streak on rich agar Petri plate in sterile conditions to get well isolated colonies. These plates can be stored in a refrigerator for about a month. Plates should be carefully examined for any contamination and if it is contaminated then it is discarded.

Spread plate/pour plate method: In spread plate methodology microbial culture is evenly distributing over LB agar plate.

- On the basis of the microbe select and prepare particular agar medium.
- To avoid condensation after pouring the plates, the autoclaved agar is cooled in the range of 45°C and 50°C. In a 100 × 15 mm media plate 15 to 20 mL of media is poured and the thickness of media is 0.3 cm approx.
- To avoid microbial contamination inoculum should be spread as soon as possible.
- Changing of pipette tips must be avoided while preparing serial dilutions to spread on the plate.

Creating Microbial glycerol stocks for long-term storage

- After inoculating the microbes for overnight in liquid culture, add 500 µL of the overnight culture to 500 µL of 40 per cent glycerol in a 2 mL cryovial and gently mix.
- Store the glycerol stock tube at –80°C. The stock will be stable for years until subjected to subsequent freeze and thaw cycles.
- Scrape off some of the frozen bacteria of the top. Do not let the glycerol stock unthaw. Streak the microbes onto an LB agar plate for overnight.

Protocol for lyophilization process

- Prepare the sterile solution—compound, mix, filter
- Fill into vials
- Partially insert a rubber closure onto the vials
- Aseptically load the vials into a freeze dry chamber
- Freeze every single solution in every vial below a pre-determined critical temperature
- Use appropriate application of temperature and pressure to sublime ice from the product
- Further apply temperature and pressure to remove the necessary amount of bound water from the product
- Automatically stopper the vials, neutralize the chamber.

Tips for maintaining strains

- Streak the culture on agar plate as soon as you receive the plate.
- Refrigerate the plate only when the good visible growth of culture is seen.
- Plates and slants are preferred over broth culture.
- Always start seed culture from single bacterial colony.
- Subculturing is preferred once in a week for maintaining broth culture.
- If the strain looks contaminated, streak and maintain fresh culture.
- Restreak the cultures on slants/plates about once a month.

Autoclaving

An autoclave is used to sterilize materials to remove all forms of microbial life with the aid of high temperature and pressure. Effective use of autoclaving is must for proper sterilization of the materials and safe use of autoclave.

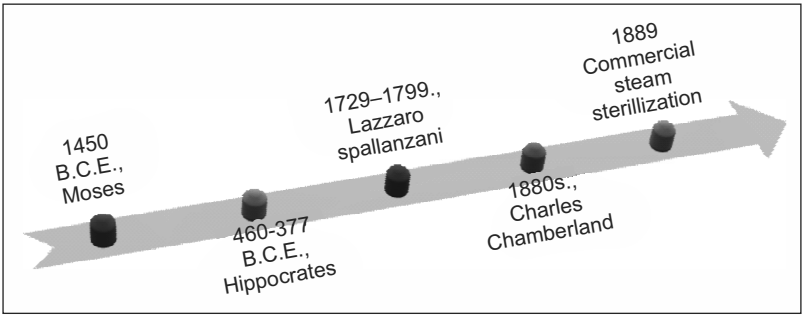
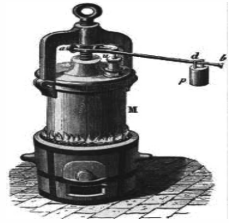


Fig. 2.1 Chronology of scientific advancement in autoclaving.

Historical significance (Fig. 2.1)

- The first sanitary code by Moses which included purification of materials by fire and boiling water.
- Hippocrates used boiling water to clean surgical instruments.
- Lazzaro Spallanzani discovered the protocol to kill bacteria in sealed glass flasks by heating it for 30 min.
- Pasteur had proposed boiling as the effective means of sterilization.
- Based on the Denis Papin invention of steam pressure cooker early design of the first autoclave was devised by Charles Chamberland.

Charles Chamberland



Papin steam cooker

Principle of autoclaving

- Steam penetrates materials in autoclave.
- Negative pressure is built due to condensation which draws in additional steam.
- Moist heat coagulates the proteins which kill the bacteria.

Standard pressure and temperature of an autoclave

When the free-flowing steam at a temperature of 100°C at pressure of 1 atmosphere above the sea-level pressure is subjected under pressure of 15 pounds pressure per square inch, the temperature inside the autoclave happens to rise up to 121°C, which is a common parameter employed in the moist heat sterilization.

Table 2.1 Some Standard Temperatures/Pressures Employed

S. No.	*Temperature (°C)*	*Pressure (psi)*
1.	115	10
2.	121	15
3.	132	27

Psi = Pressure unit standing for pounds per square inch

Pre-Autoclave Procedures

(i) Polypropylene bags

They are autoclave or biohazard bags which are rigid, tear-resistant and available in various sizes. They should not be fully filled and some space should be left at the mouth region for passage of the air. Indicator tape should be placed in an "X" pattern over the biohazard bag.

(ii) Materials that can be autoclaved

- Plastic tubes, pipette tips and plates
- Glassware
- Surgical Instruments
- Biohazardous waste

(iii) Risk Management

Potential risks while operating autoclave

- Heat burns
- Steam burns
- Heat fluid scalds
- Hand and arm injuries due to door
- Body injury if there is any explosion

(iv) Health and Safety

- Trained person should be appointed as incharge of the autoclave.

- Autoclave should be operated only after wearing protective clothing.
- Autoclave must be inspected annually and proper record be maintained for its inspection, service and repairs.

(v) Personal Protective Equipment for Users

- Eye protection
- Heat-resistant gloves
- Lab coat, buttoned
- Closed-toed shoes

(vi) Loading Autoclave

- Place material in autoclave
- Do not overload
- The door should be firmly latched and closed

(vii) Steps to Autoclave

- Check that the water reservoir is filled.
- Set appropriate temperature for the cycle.
- Close and lock door.
- Pressure is achieved in 40 min approx. If autoclave is cold and 20 min approx. if in use.
- To STERILIZE turn on the autoclave.
- The autoclave should be turned to VENT when the cycle is complete, and door must remain closed until pressure drops. During venting temperature stays on for longer time and the steam present in chamber prevents melting of plastics.
- Turn off the autoclave.

Unloading Autoclave

- Heat-insulating glove provides protection against burns.
- Before opening the door one must ensure that the pressure of chamber is '8 psi'.
- While opening the door one should wear gloves and keep distance from the door.
- Super-heated liquid in containers should not be opened to avoid accidents.
- Autoclaved hot items should be placed in a room to cool the temperature.

derived from those organisms; and allergens and toxins derived from higher plants and animals.

Biosafety: The containment principles, technologies and practices that are implemented to prevent unintentional exposure to pathogens and toxins, or their accidental release.

FREQUENTLY ASKED QUESTIONS

1. What is the best way to preserve cultures for long?
2. Are there special requirements for reconstituting certain lyophilized microorganisms?
3. What should we do with contaminated plates?
4. How long should we sterilize the liquids?
5. Why contamination issues are observed even after autoclaving?

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