Sterilisation of microbiological waste

Posted by Anonymous on Fri, 2018-08-03 16:53

Sterilisation of microbiological waste: Science ASSIST has received a number of questions, which all relate to this topic, we will cover all of these in this question.

Q1. What does sterilisation mean and what are the correct conditions for sterilisation to occur in an autoclave/pressure cooker? Can I use a kitchen pressure cooker to sterilise my microbiological waste?

Q2. Can I sterilise microbiological waste in an autoclave bag with a biohazard symbol then put it in the general waste bin?

Q3. Can I use an oven bag instead of an autoclave bag? What is the correct procedure for using an autoclave/oven bag? Is it ok practice to place inoculated agar plastic Petri dishes into an autoclave/oven bag to be sterilized, even if they melt?

Q4. How are contaminated liquid cultures sterilised?

Q5. Is it ok to open glass Petri dishes, or polycarbonate jars after sterilisation, scrape out the waste and then wash and reuse them.

Q6. Taping around Petri dishes with parafilm to prevent re-opening - will this prevent steam entering the dish and therefore sterilisation?

Q7. What type of indicator strips can be used to validate the sterilisation process?

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Year Level:
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Senior Secondary
Laboratory Technicians:

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Autoclaving microbiological waste

Submitted by sat on 03 August 2018

Answer reviewed 24 January 2023

Science ASSIST has developed a comprehensive document called Guidelines for Best Practice for Microbiology in Australian Schools see GUIDELINES for best practice for microbiology in Australian schools. We strongly recommend you download this document as it discusses in detail the underpinning knowledge and laboratory techniques required for schools to successfully prepare, deliver and disassemble microbiology practical activities.

In addition, Science ASSIST has other related resources such as

- **SOP Operating a pressure cooker and autoclave**
- **AIS: Decontaminating microbiological equipment**
- **AIS: Sterilising agar**

Sterilisation

Sterilisation is a process that destroys all viable microorganisms, including highly resistant bacterial endospores. All microbiological waste is required to be sterilised using a pressure cooker or autoclave before disposal.

Sterilisation is accomplished by using moist heat in the form of steam under pressure at high temperatures, i.e., 121°C for 15-30 minutes at 15 psi (pounds per square inch of pressure). It is imperative that timing only begins when the temperature and pressure conditions have been reached to guarantee that sterilisation has occurred. This method will denature & coagulate enzymes and other cell constituents in the bacterial cell including any spore formers. Fungal cultures including fungal spores are easily killed by heating above 80°C.

A laboratory pressure cooker, (pressure steam steriliser) or an autoclave is commonly used in the laboratory to effectively sterilise microorganisms and agar and is the preferred method for sterilisation in the school laboratory.

Note: you should not put anything soaked in chemicals such as 70% alcohol or sodium hypochlorite through an autoclave/pressure cooker, because of the potential for toxic gases to be produced.

Efficient methods of decontamination and sterilisation in school laboratories are outlined in Science ASSIST: *SOP: Operating a pressure cooker and autoclave*, see https://assist.asta.edu.au/resource/4755/microbiology-sops- -school-level-2

Pressure cookers suitable for school science laboratories

It is recommended that a pressure cooker used for the sterilisation of agar and microbiological waste be equipped with a pressure gauge that indicates the pressure and temperature within the unit. When considering purchase of a laboratory pressure cooker:

- The pressure cooker **must** contain a pressure gauge and be able to reach a pressure of 15 psi (103 kPa) and a temperature of 121° C to ensure items and waste are sterilised.
- Ensure the pressure cooker is an adequate size for the items to be sterilised. Space is required around items for steam to circulate.

It should be kept in mind that many domestic kitchen pressure cookers **do not** meet these requirements. For further information on the use of pressure cookers and sterilising see the following resource material developed by Science ASSIST, AIS: Sterilising Agar and SOP: Operating a pressure cooker and autoclave.

Laboratory pressure cookers specifically designed for use in laboratories are available from various scientific suppliers. See the Science ASSIST School science suppliers list.

Autoclave/oven bags

It is recommended to sterilise non-liquid microbiological waste by containing the waste in a bag that will withstand the sterilisation conditions of 15psi, 121^0C for 15-30 minutes and contain the treated contents.

Schools have the option of using two different types of bags:

- Autoclavable biohazard bags which are available from scientific suppliers, see the Science ASSIST School science suppliers list. These are made from a heavy duty plastic e.g. polypropylene marked with the international biohazard symbol and usually have the word autoclavable written on them. They are available in a variety of sizes.
- Oven bags which can be purchased from supermarkets and which can also withstand the sterilization conditions. These are also available in different sizes, do not have any biohazard markings on them and are commonly used in schools. It may be necessary to use two combined oven bags to minimize any chance of them splitting

It must be remembered that effective sterilisation is also determined by the correct packing of an autoclave or oven bag. The effectiveness of sterilisation depends on steam being able to penetrate what is being autoclaved.

Correct procedure for using an autoclavable biohazard or oven bag for sterilising non-liquid microbiological waste in either an autoclave or laboratory pressure cooker:

- **Loosely pack non-liquid microbiological waste including agar plates into bags to no more than 2/3 full** to ensure that the steam during sterilisation is allowed to penetrate the entire load.
- **Make sure there are no sharp objects present** that may puncture the bag.
- **Loosely tape shut the bag leaving an opening of at least 5–6cm** to allow good steam penetration.
- **Place the bag into a secondary container** within the steriliser to prevent any leakage should the bag rupture.
- **Do not overload the steriliser** with too many bags as this may block steam circulation.
- **Use a sterility compliance strip** placed in the centre of the load to verify sterilisation conditions have been reached.
- **Sterilise at 15psi, 121^{O}C for 15–30 minutes.**
- After sterilisation has been verified, the autoclave/oven bag containing waste items should be **disposed of by placing it into a sturdy garbage bag which is sealed for immediate disposal in the general waste bin. Note: if using bags with a biohazard symbol on them, this symbol should not be visible through the (secondary) garbage bag to avoid any undue alarm or concern.**
- **Wear heat protective gloves and a face shield** when removing waste from the steriliser.
- **Sterilisation ideally takes place as soon as possible** after completion of a practical activity and should occur within the preparation room.

How are contaminated liquid cultures sterilised?

Liquid cultures in bottles or test tubes can be placed into a secondary container in the autoclave/pressure cooker with their lids loose and sterilised at 121°C for 15-30 minutes at 15 psi. **It is important that lids remain loose to allow steam penetration and prevent the build-up of pressure that may lead to bottles or tubes exploding**.

Is it ok to open Petri dishes after sterilisation, scrape out the agar waste and then wash and reuse them?

Plastic Petri dishes are commonly made from clear polystyrene plastic which are heat resistant up to 80°C[1]. The best way to sterilise plastic Petri dishes containing agar inoculated with microorganisms is by placing them into an autoclave or oven bag that will withstand the sterilisation conditions in an autoclave/pressure cooker.

Plastic Petri dishes are regarded as a single use disposable item and under sterilisation conditions will deform, the agar will melt and run out of the deformed dishes collecting in the autoclave/oven bag which can then be disposed in the general waste.

Glass Petri dishes containing agar inoculated with microorganisms OR polycarbonate tubes/jars used for plant tissue culture, can also be sterilised by placing them into an autoclave or oven bag in an autoclave/pressure cooker. After sterilisation the glass Petri dishes/polycarbonate tubes/jars and their contents would be regarded as sterile and are safe to open, scrape out any residual agar back into the autoclave/oven bag and then be washed

with warm soapy water, rinsed, dried and sterilised for re-use.

If Parafilm is used to tape around Petri dishes will it stop steam entering the dish and prevent sterilisation?

Petri dishes sealed with sticky tape placed at 12 o'clock, 3 o'clock, 6 o'clock and 9 o'clock or with a **single layer** of Parafilm should be placed into the autoclave/oven bag with the tape/Parafilm left on. It will not prevent sterilisation.

Plates can be sealed around the whole circumference with laboratory sealing film such as Parafilm® M after incubation but before viewing to reduce the risk of students opening the plates and to reduce the loss of any liquid that has accumulated in the plate.

Parafilm M becomes more flexible, softer and stickier at about 54°C and has a melting point of 60°C [2]. So in an autoclave/pressure cooker at 121°C it will have melted from around the Petri dish no longer forming a seal, therefore allowing sterilisation to occur as steam is allowed to enter the vessel. In addition, the moisture in the agar would also convert to steam to help facilitate the sterilisation process.

Indicator strips used to validate the sterilisation process

In a school setting Class 5 integrated chemical indicator strips are suitable to validate the effective operation of an autoclave/pressure cooker. These strips are the most accurate of the internal chemical indicators and are considered comparable to Biological Indicators in saturated steam[3]. They react to the critical parameters of temperature and steam and have the advantage of not requiring any incubation, as an immediate result is produced.

An indicator strip should be placed in the centre of each load and checked after each run to ensure that the temperature and steam conditions have been met.

Biological indicators – Traditionally the most accepted means of sterilisation monitoring. They contain spores of a heat resistant bacterium such as *B. stearothermophilus* that will germinate if the correct sterilisation conditions are not met. This microorganism is the most resistant strain to steam autoclaving and will be inactivated under correct autoclave conditions. Biological indicators require an incubation step to obtain a result. Professional microbiology labs would regularly test their autoclave using this method. **This would not generally be used in a school setting.**

Both biological and chemical indicators are available from scientific suppliers. See the Science ASSIST School science suppliers list.

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