Agar plate setting conditions, storage, inoculation and disposal

Posted by Anonymous on Thu, 2018-09-06 16:31

Agar plate setting conditions, storage, inoculation and disposal: What are the factors that affect the ability of agar to set? Is it necessary to store agar plates in the fridge and if so for how long? What is the correct procedure for labelling, inoculating, sealing and disposal of agar plates that contain live microbial cultures in school science?

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Agar plate storage in the fridge

Submitted by sat on 06 September 2018

Answer updated 18 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.

Setting of agar plates:

The ability of agar to set is affected by the concentration of the agar, the sugar content and the pH. Varying the pH of the agar too far (more than 1 unit) away from neutral (pH 6–8) could result in the agar not setting. In an acidic pH range, this is due to the hydrolysis, or chemical breakdown of the agarose polysaccharides, the units that bind together to form the agar gel. In addition, the effect of pH on the setting process is more evident after heating. Agar liquifies at 100°C and solidifies at around 42°C.

Agar plates when poured should be allowed to set undisturbed at room temperature. It is best practice when the plates are completely set and cooled to room temperature that they are stored upside down in sealed plastic bags in the fridge at 4 °C. See SOP Preparing nutrient and plain agar plates https://assist.asta.edu.au/resource/4755/microbiology-sops-updated-school-level-2. It is a good idea to reuse the plastic bags from which the sterile Petri dishes came from for storage. Storage this way will prevent:

- Condensation that develops on the lid from dripping onto the agar surface which is a potential source of contamination.
- Moisture loss which leads to the agar drying out.
- Chemical degradation from exposure to light and heat.

Storage of prepared agar plates:

The type of agar used will determine the length of time that it can be stored in the fridge[1]. The recommended agar for use in schools is a general all- purpose nutrient media such as Nutrient agar. This type of agar plate should be stored at 4 °C and used within a month of preparation to prevent it drying out. Loss of moisture can be visually detected by the appearance of shrinkage away from the plate and macroscopic cracks that develop in the agar. Agar plates which are poured too thin will also dry out faster.

Labelling agar plates:

Petri dishes should always be labelled on the base containing the media so that if the lid is inadvertently changed the identification of the culture is still known. Information should be written around the perimeter of the base to avoid covering the colonial growth[2].

Inoculation techniques commonly used in schools:

Microorganisms can be inoculated onto agar plates in school science laboratories by various methods.

1. Inoculation of agar plates via air exposure (settle plates)
2. Inoculation by direct contact of the surface of the agar with an item such as a pen lid.
3. Inoculation of agar plates with sterile swabs from environmental surfaces
4. Inoculation of agar plates with bacteriological loops or sterile swabs from water or food samples such as yoghurt or cheese.

5. Inoculation of agar plates with a risk group 1 microorganism (RG1) using pipettes and glass spreaders to produce a bacterial lawn culture for testing antimicrobial substances.
6. Inoculation of agar plates with a risk group 1 microorganism (RG1) using the streak-plate method to gradually dilute an inoculum of bacteria over the surface of an agar plate to produce single isolated pure colonies.

Note:

- When sampling from the environment, the microorganisms collected are unknown, so it is important to sample from environments that are not likely to contain human pathogens. These cultures are considered "wild cultures" and should **not be opened or subcultured**.
- School science laboratories are classified as (PC1) Physical Containment level 1, if they comply with the requirements of AS/NZS 2243.3-2022. Safety in laboratories. Part 3. Microbiological safety and containment. PC1 laboratories are suitable for work with risk group 1 (RG1) microorganisms only. These are infectious microorganisms that are "*unlikely to cause human or terrestrial animal disease*" and "*where laboratory or facility personnel can be adequately protected by standard laboratory practices and equipment*" [3].

Subculturing:

The aseptic transfer of microorganisms from one medium to another is a specialised technique requiring sound knowledge and expertise to minimise the risks involved. It is a skill developed with much practice. This procedure is not permitted in some jurisdictions. You should check the activities permitted in your jurisdiction before proceeding. Teachers supervising students carrying out these activities should be highly trained in microbiological techniques.

Sealing agar plates:

The complete sealing of Petri dishes during incubation is not recommended, as this may generate an anaerobic environment inhibiting the growth of aerobic microorganisms and promoting the growth of potentially anaerobic pathogens. The growth of anaerobic microorganisms, those that cannot be grown in the presence of oxygen, should be avoided in school laboratories. Anaerobes are widely distributed in nature and are potentially pathogenic (capable of causing disease) when removed from their normal environments[4]. Most anaerobes fall under Risk Group 2 or 3 microorganisms which are not to be handled in PC1 laboratories.

Science ASSIST recommends that the lid and base of the Petri dish be taped with 4 pieces of sticky tape [2, 5] to allow for aerobic conditions and to prevent accidental opening of the plate during incubation. Alternatively, one piece of laboratory sealing film e.g., Parafilm® M that is cut no wider than 1cm may be wrapped **once** only around the circumference of the agar plate to allow adequate gas exchange. Gas permeability data for Parafilm® M indicates that when used as a **single layer it will allow sufficient oxygen exchange** to promote the growth of aerobic microorganisms and inhibit the growth of potential anaerobes. Wrapping Parafilm

around a Petri dish **more than once should be avoided** as it will be sufficient to stop any gas exchange creating an undesirable anaerobic environment.

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

Incubation of agar plates:

In the school laboratory, to reduce the risk of growing pathogens, agar plates and broths should be incubated between **room temperature (22–25°C) and a maximum of 30°C for no greater than 30-36 hours.** Agar plates must be incubated upside down to prevent the condensation that occurs on the lid to drip onto the culture. Condensation will interfere with colonial growth and may introduce a contaminant. Most cultures suitable for use in schools will grow at room temperature and can be incubated satisfactorily in a cupboard. An incubator where the temperature is controlled may also be used to incubate agar plates or broths as a constant temperature can be maintained.

Storage of inoculated agar plates:

Following incubation, storage of inoculated plates at 4 °C will slow down the growth of cultures allowing you to show students a 2-3 day growth if lessons are a week apart. Store plates upside down wrapped in plastic to prevent condensation from the lid dropping onto the agar surface (a potential source of contamination) and causing isolated colonies to spread into each other.

Disposal of agar plates:

Agar plates should be sterilised before disposal. When observations are complete, the plates should be placed into an autoclave or oven bag and sterilised in an autoclave or pressure cooker at 110 kPa/15psi, 121° C for 15–30 minutes before disposal into the general waste bin. See AIS: Sterilising agar and SOP: Operating a pressure cooker and autoclave

Procedures to prevent the growth of pathogens:

In addition to not completely sealing agar plates during incubation, there are other procedures that should be carried out in school laboratories in order to prevent the growth of pathogenic organisms.

- When handling microorganisms, it is important to use aseptic techniques at all times. A significant risk associated with microbiology is the generation of microbial aerosols, where fine droplets of water containing cells and/or spores are released into the air.
- Aseptic technique is a fundamental skill in microbiology:
    - to avoid the contamination of culture media with unwanted microbes,
    - to prevent contamination of personnel and work surfaces, and
    - to prevent microbes from being accidently released into the environment.
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The type of media used should not select for pathogens.
    - **Nutrient agar** is a simple media which supports the growth of a wide variety of bacteria and moulds and is suitable for use in school laboratories.
    - Media designed to select for more fastidious microorganisms and pathogens such as Blood and MacConkey Agar **should not** be used.
- When culturing from the environment, samples **should not** be taken from areas likely to contain organisms harmful to humans, for example: body surfaces, coughs, sneezes, animal sources such as bird cages and unsanitary environments such as drains and toilets.
- Cultures should be incubated at **temperatures of 30° C or below** to avoid the growth of potential human pathogens that are adapted to human body temperature.

Precautions when inoculating agar plates:

- Wash hands with soap and water before and after working with microorganisms.
- Cover any cuts with a waterproof dressing and consider wearing disposable gloves.
- Make sure work surfaces are decontaminated before and after working with microorganisms with 70% ethanol.
- Make sure inoculating instruments (loops, swabs, pipettes and spreaders) are sterilised prior and after use.
- Make sure that inoculating instruments containing microbiological samples are not allowed to touch any surface other than the agar that requires inoculation.
- Flame the mouth of all test tubes and bottles both when the cap is removed and before it is replaced.
- Plates should be open for a minimum amount of time to minimise the risk of introducing any contaminants from the air.
- Inoculation should be carried out as quickly as possible to minimise introducing any contaminants.
- Work close to the Bunsen flame, as it provides an updraught that carries air away from the workspace, so reducing contamination from the air.
- Have a bacterial spills kit available (freshly prepared 1% sodium hypochlorite, bucket, mop, plastic waste disposal bags, paper towel, disposable gloves, safety glasses, mask, plastic disposable apron).

It is important that microbiology activities are supervised by staff who are aware of the safety procedures required in dealing with biohazards. Science ASSIST recommends that a risk assessment is carried out and appropriate control measures be put into place.

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[3] AS/NZS 2243.3:2022, *Safety in Laboratories, Part 3: Microbiological safety and containment* This excerpt is reproduced by ASTA with the permission of Standards Australia Limited under licence CLF1222asta. Copyright in AS/NZS 2243.3:2022, *Safety in Laboratories, Microbiological safety and containment* vests in Standards Australia and Standards New Zealand. Users must not copy or reuse this work without the permission of Standards Australia or Standards New Zealand or the copyright owner.

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