Agar plate experiments

Posted by Anonymous on Mon, 2014-12-22 13:09

Agar plate experiments: Can E. coli and/or Staph epidermidis be cultured from a broth onto an agar plate in a classroom situation. I note that both these cultures can be commercially bought and are labelled as 'Bacteria-Risk Group 1 Suitable for Schools'. I had thought subculture of bacteria was not allowed, only primary exposure of plates, then sealing. The experiment requested involves putting the known culture onto plates then placing discs of antiseptics/disinfectants or antibiotic mast rings onto the plate to demonstrate their effectiveness.

Voting:

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Year Level:

7
8
9
10
Senior Secondary

Laboratory Technicians:

Laboratory Technicians

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Answer by labsupport on question Agar plate experiments

Submitted by sat on 19 January 2015

There are many different considerations in a school laboratory regarding the use of microorganisms. Science ASSIST is currently consulting authorities in order to make nationally consistent sensible and workable recommendations for best practice in school microbiology. In the meantime, we understand that you need some direction on this matter. The activity you are describing contains a number of risks and we recommend careful consideration of the many safety issues before proceeding with this activity.

Generally, school science laboratories are classified as Physical Containment level 1 (PC1), **if** they conform to the requirements specified in Section 5 of AS/NZS 2243.3:2010 Safety in Laboratories – Microbiological safety and containment. If they conform to these requirements, then they are only suitable for work with microorganisms where the hazard levels are low, and where laboratory or facility personnel can be adequately protected by standard laboratory practice.[i] Microorganisms that are classified as Risk Group 1 are the only ones that should be used in PC1 laboratories. Higher levels of Physical Containment are required for handling fresh human tissues or body fluids and microorganisms of Risk Groups 2–4[ii].

When carrying out this type of sensitivity test on a microorganism, a lawn culture needs to be produced from a pure broth culture of the organism. The broth culture is either purchased as a live broth or prepared by emulsifying several colonies from a plate culture in a sterile broth in a test tube to a particular density. Then, using a sterile swab, a sample is inoculated over the entire surface of an agar plate. Antibiotic or disinfectant impregnated discs are applied to the inoculated surface and the plate incubated. Sensitivity or resistance is determined by observing zones of inhibition around the discs. Although the cultures that you mention are commercially available and classified as Risk Group 1 there are several aspects to consider regarding this activity.

- **Risks:** It should be remembered that even though the microorganisms are from Risk Group 1, some can still pose a low level of risk to the community as they can be capable of causing disease if provided with appropriate conditions. People who are immuno-suppressed are at greater risk.
- **Jurisdictional Policies:** There are differences between the state and jurisdiction policies regarding whether any manipulation or subculturing of microorganisms is allowed. For example, subculturing of pure cultures is permitted in some jurisdictions subject to following strict safety guidelines. However, it is not permitted in WA Department of Education schools and so schools should check if subculturing is allowed in their jurisdiction.
- **Training:** It requires good microbiological training to have an appropriate level of understanding and technical expertise to apply correct aseptic techniques when manipulating microorganisms. The majority of teachers and lab techs may not have any formal training in microbiology and may not be able to tell if a culture is contaminated and/or contains pathogens and consequently the wrong microbes may be cultured.
- **Aseptic Technique:** When dealing with any microbiological culture, and in particular live broth cultures, the use of aseptic techniques to prevent aerosol production is important. Release of microorganisms in the form of aerosols increases the risk of infection by inhalation.
- **Contamination of the culture:** It is important to have a pure culture of the microorganism. Aseptic technique is a fundamental skill in microbiology to maintain pure cultures whilst subculturing, to prevent microbes from being accidently released into the

environment and infecting others in the laboratory. In a school, teachers and laboratory staff are only able to determine visually if a culture is pure, mixed or contaminated. It is easier to observe if an agar plate contains a pure, mixed or contaminated culture and it is more difficult to be sure that a liquid culture contains a pure culture of microorganisms. Some jurisdictions may allow gram stains to be performed to check for purity of a culture. If there is any doubt about the purity of the bacterial sample, it should not be used.

- **Subculturing:** when subculturing a microorganism from plate to plate the number of subcultures needs to be limited as excessive subculturing increases the risk of phenotypic alteration.
- **Waste disposal:** It is essential that appropriate waste disposal and sterilisation* procedures are adhered to, an appropriate spill kit is available to handle any spill and that staff are trained in its use.
- **Logistics:** If this activity were to be run in the classroom situation, it would require the purchase or aseptic preparation of numerous tubes of sterile broth, sterile agar plates, sterile swabs and sterile forceps to apply discs. There are logistical constraints of a laboratory technician with regard to time allocation, training and expertise in microbiology techniques to prepare for a classroom situation as well as the fact that school facilities are not generally set up for this level of microbiology.

*Science ASSIST recommends the use of a pressure cooker or autoclave for sterilising rather than chemical sterilisation, which has risks and limitations. For information regarding sterilising agar see AIS: Sterilising Agar.

Here are some additional links to safety considerations on this topic

https://www.sciencebuddies.org/science-fair-projects/project_ideas/Micro_Safety.shtml

https://assist.asta.edu.au/resource/4196/guidelines-best-practice-microb...

http://sydney.edu.au/whs/guidelines/biosafety/decontamination_guidelines.shtml

https://microbiologyonline.org/teachers/safety-information

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[i] University of Sydney. 2013. Biological Safety – Microbiology http://sydney.edu.au/whs/guidelines/biosafety/microbiol.shtml (accessed July 2014)

[ii] Australian Standards AS NZS 2243.3-2010. Safety in Laboratories – Microbiological safety and containment

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