Antimicrobial Sensitivity Testing

Posted by Anonymous on Wed, 2016-02-10 11:41

Are schools allowed to conduct antimicrobial susceptibility testing of a microorganism using the Kirby-Bauer disc diffusion method? What antimicrobials are allowed for use in schools? Are antibiotics permissible?

Voting:
No votes yet

Year Level:
Senior Secondary
Laboratory Technicians:
Laboratory Technicians

Showing 1-1 of 1 Responses

Use of antibiotics (Amoxycil) in experiments

Submitted by sat on 16 February 2016

Answer reviewed 15 February 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.

School policies

There are differences between the state/territory educational jurisdiction policies on whether certain microbiological activities can be carried out. Schools are advised to check what activities are permitted in their jurisdiction/school sector before proceeding to work with any microorganisms.

Antimicrobial sensitivity testing using the Kirby-Bauer disc diffusion method requires lawn cultures to be produced1. This requires subculturing, which is a specialised technique requiring sound knowledge and expertise to minimise the risks involved. Some jurisdictions do not allow microorganisms to be subcultured.

Antimicrobial sensitivity testing:

The susceptibility or sensitivity of a microorganism to antimicrobials (antibiotics, antiseptics or disinfectants) can be conducted using a variety of methods. Common antibiotic sensitivity methods include dilution techniques, disc diffusion methods, Etests and some automated testing systems. These techniques are generally used in pathology, food technology and other industrial laboratories. The Kirby-Bauer disc diffusion method is the most common technique used in school science.

Disc diffusion method:

An antimicrobial which is incorporated into a small paper disc is placed onto an agar plate containing a lawn culture of the microorganism to be tested. A lawn culture is produced from a pure broth culture of the test microorganism. The broth culture is either purchased as a live broth or prepared by emulsifying several colonies from a plate culture in sterile broth to a particular density. A sample of this broth culture is inoculated over the entire surface of an agar plate either with a swab stick or an L-shaped spreader (hockey stick). Antimicrobial impregnated discs are applied to the inoculated surface and the plate incubated. The antimicrobial agent diffuses into the agar resulting in either sensitivity or resistance which is determined by observing the presence/absence of zones of inhibition around the discs.

Suitable antimicrobials for use in schools:

Antimicrobials suitable for this activity in school science include:

- disinfectants such as household cleaners, e.g. Pine O Clean
- antiseptics such as Dettol

The use of commercially prepared antibiotic discs in school activities is not recommended due to staff/student allergies and the potential for antibiotic resistance.

- Any antibiotic, including antibiotic discs, should not be handled if the person is allergic or sensitive to that particular antibiotic or class of antibiotics. E.g. amoxicillin which belongs to the class of antibiotics called penicillins.
- Overuse or misuse of antibiotics can lead to the development of resistant microorganisms.
- If it is deemed appropriate to use antibiotics, then there are a wide variety of antibiotics and concentrations, available in disc form from various scientific suppliers. These are safer than an antibiotic in solution and can be readily handled wearing appropriate PPE with sterile forceps to avoid any skin contact.
- Safety data sheets should always be consulted when using any chemicals.

Science ASSIST has produced an SOP: Susceptibility testing of antiseptics and disinfectants SOP: Susceptibility testing updated Jan 2023 and an SOP: Preparing a bacterial lawn culture SOP: Preparing a bacterial lawn culture updated Jan 2023

Science ASSIST recommendations:

Antimicrobial sensitivity testing aligns with School Level 3 of the Science ASSIST Microbiology Guidelines. School work level 3 is considered low to medium risk due to the growth of pure cultures of Risk Group 1 microorganisms and the use of certain manipulations such as subculturing.

Science ASSIST recommends that if schools decide to go ahead with this type of activity that a detailed biological risk analysis and assessment needs to be conducted taking the following into consideration:

- Correct facilities are in place: The lab must be a PC1 laboratory. School science laboratories are generally 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.
- Staff Training: Teachers and technicians should be highly trained and experienced in good microbiological laboratory practice to be aware of and safely manage all risks. Additional staff are recommended to enable a high level of student supervision.
- Microorganism used: Only Risk group 1 bacteria (RG1) obtained as pure cultures from reputable biological suppliers should be used. RG1 microorganisms are unlikely to cause human disease in healthy individuals. However, these are still considered opportunistic and may be a risk to people who are immunocompromised or immunosuppressed.

Manipulations:

- The type of media used should not select for pathogens (e.g. selective or enriched agars). Nutrient agar is a simple medium which supports the growth of a wide variety of bacteria and moulds and is suitable for use in school laboratories.
- The incubation temperature recommended for schools is < 30° C. This temperature does not encourage the growth of pathogens potentially harmful to humans.
- Following incubation, plates should be sealed with Parafilm or similar to prevent students being able to open the plates when observing them.
- After inoculation and during the preparation for incubation, the plates should be kept closed using either four pieces of tape or a single layer of Parafilm to allow for aerobic conditions. These plates should never be reopened after incubation.
- Following incubation, plates should be sealed with Parafilm or similar to prevent students being able to open the plates when observing them.
- Students should never subculture from cultures that they have inoculated because of the high risk of contamination with unknown microorganisms.
- Aseptic techniques should be used at all times to minimise the production of microbial aerosols.

Hygiene practices:

- Hands should be thoroughly washed with soap and water before commencing the activity and on leaving the laboratory.
- Appropriate PPE should be worn at all times Cuts or wounds on the hands should be covered with a waterproof dressing or Band aid.
- Hands should be kept away from the mouth, nose, eyes and face during and after this activity.
- Workbench surfaces should be disinfected with 70% v/v ethanol or hospital grade disinfectant before and after microbiological activities.

Staff/student health:

Determine if any staff or students who will be close to the activity, have an allergy to antibiotics if being used or are immunosuppressed?

Waste disposal:

All waste must be sterilised prior to disposal using an autoclave or laboratory pressure cooker at 15psi, 121^{o} C for 15-30 minutes.

References:

1 Hudzicki, J. (2009). 'Kirby-Bauer Disk Diffusion Susceptibility Test Protocol', Retrieved from The American Society for Microbiology website (15 February 2023) https://asm.org/Protocols/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test...

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