Science ASSIST
Standard
Operating
Procedure

Susceptibility testing of antiseptics and disinfectants

1. Introduction

Antiseptics are used in the disinfection of external surfaces of living tissue. Disinfectants are used to disinfect inanimate objects such as equipment and bench surfaces. This activity uses the Kirby-Bauer disc diffusion method of antimicrobial sensitivity testing. In this test an antiseptic or disinfectant is incorporated into a small paper disc, which is placed on an agar plate containing a pure lawn culture of the microorganism to be tested.

To check if an antimicrobial agent can control a microorganism, it must be tested together with the growing microorganism to see what effect it may have on it.

The antiseptic or disinfectant diffuses into the agar and can inhibit the growth of the organism if it is **sensitive** to the test agent, producing a clear zone of inhibition or no growth. Sensitivity is a term used to describe **effective** antimicrobial control.

Absence of a zone of inhibition is indicative of resistance which occurs when an antiseptic or disinfectant is not able to kill or inhibit the growth of the microorganism. Resistance is a term used to describe **ineffective** microbial control.

Any zones of inhibition are measured after incubation and these values can be compared to an interpretation table with standardised data. Doctors use Kirby-Bauer test results to choose antibiotics effective against the specific bacteria causing a patient's infection.

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

2. Context

- This activity aligns with **School Level 3** of the Science ASSIST Microbiology Guidelines.
- This practical activity should be supervised by science teachers and technicians who are highly trained in microbiological techniques. Staff need to be trained and experienced in aseptic technique, subculturing, recognising contamination and sterilisation and decontamination procedures.
- PC1 facilities required. Autoclave or pressure cooker with gauge required for sterile preparation of media, equipment and for decontamination.
- Microorganisms (Risk group 1 bacteria) obtained as pure cultures from school biological suppliers Additional staff are recommended to enable a high level of student supervision when undertaking this activity.

3. Safety Notes

To be undertaken only by trained personnel in conjunction with a current Safety Data Sheet (SDS) and site-specific risk assessment. Before proceeding, ensure that this activity is permitted within your school jurisdiction.

- Do not commence work unless a site specific risk assessment is completed and control measures are implemented. Immunosuppressed staff and students must be considered.
- Regard all microorganisms as potential pathogens and treat them with standard microbiological practices to minimise risk to laboratory staff and the environment.
- Use only a pure culture of a Risk Group 1 (RG1) microorganism.
- No subculturing from plates or broths inoculated by students.

- Observe good hygiene practices and aseptic techniques at all times.
  - Wash hands thoroughly with soap and water before commencing activity and on leaving the laboratory.
  - Cover any cuts or wounds on the hands with a waterproof dressing or Band aid.
  - Keep hands away from the mouth, nose, eyes and face during and after this activity.
  - Decontaminate workbenches with disinfectant (70% v/v ethanol or hospital grade disinfectant) before and after microbiological activities.
  - Keep 70% v/v ethanol away from heat and sources of ignition.
  - Use aseptic techniques to minimise the production of microbial aerosols.
  - Conduct all work close to a Bunsen flame.
  - Only open Petri dishes, tubes and bottles for the minimum amount of time
- Waste must be sterilised before disposal at 15psi, 121˚C for 15-30 minutes.
- Use caution when working with a Bunsen burner, the neck of culture tubes may be hot.
- Disposable plastic aprons are not recommended when Bunsen burners are used.
- Care must be taken if there is a great deal of condensate in the lid of the agar plate. This can easily drip out carrying microorganisms with it.
- Count plates out and in.
- Follow all general laboratory safety guidelines and wear appropriate PPE.

4. Regulations, licences and permits

Consult the science guidelines in your jurisdiction for policies regarding the subculture of microorganisms.

5. Equipment

- PPE: safety glasses, laboratory coat; non-slip, non-porous closed in shoes.
- 70% v/v ethanol or hospital grade disinfectant and paper towel
- Beaker to collect contaminated forceps to be sterilised in an autoclave or pressure cooker. Do not place metal forceps into bleach as they will rust.
- Nutrient agar plates pre-inoculated with a bacterial lawn culture. See SOP: Preparing a bacterial lawn culture
- 4 pairs of sterile forceps (in test tubes covered with foil)
- Sterile paper discs (available from biological suppliers)
- Suggested antiseptics and disinfectants: liquid handwash, various concentrations of alcohol, 1% iodine solution, Dettol diluted 1:20, hospital grade disinfectant prepared according to the manufacturer's recommended effective concentration. Use no more than 4-5 discs per plate.
- Bunsen burner
- Sticky tape or laboratory sealing film (e.g., Parafilm® M)
- Incubator or suitable area for incubation that is away from sunlight and between room temperature (22-25˚C) and a maximum of 30˚C.

6. Operating procedure

1. Prepare the laboratory by closing all windows and doors to prevent draughts and the entrance of staff and students.
2. Remove all non-essential books and equipment from the workspace and disinfect the work bench with 70% v/v ethanol or hospital grade disinfectant and paper towel. Allow alcohol to evaporate ensuring all alcohol vapours have dissipated before lighting Bunsen burners
3. Collect all equipment required including lawn inoculated nutrient agar plates, sterile forceps, sterile paper discs and antiseptics and disinfectants for testing
4. Using a permanent marker, divide the base of the agar plate into 4 sections. Label the edge of each section as Control, and 3 selected antiseptics/disinfectants
5. Light the Bunsen to create a hot blue flame which provides an updraft of warm air for the surrounding area to help reduce contamination.
6. Working close to the Bunsen flame and using sterile forceps, aseptically pick up a blank sterile disc and place the edge of it in the first chemical agent to be tested. Allow the solution to wet the whole disc by capillary action. Do not over saturate the disc. Lift the lid of the agar plate and place the wet disc on the inoculated agar plate in the appropriate labelled section. Immediately put the lid back onto the agar plate.
7. Repeat step 6 each time with a new sterile pair of forceps for the other antiseptic/disinfectant agents. Place used forceps in a discard container (beaker) to be decontaminated in the autoclave at the end of the activity.
8. Tape the agar plate closed with 2–4 short pieces of sticky tape to hold the lid on to the base or with one strip of laboratory sealing film wrapped once around the circumference of the agar plate.
9. Incubate the Petri dish upside down for 24–48 hours between room temperature (22-25˚C) and a maximum of 30˚C in an aerobic environment. This can be in an incubator or an area in the laboratory away from direct sunlight. The moisture on the disc will hold it in place. If the discs are over saturated, they may move over the surface of the agar.
10. Decontaminate the bench with 70% v/v ethanol.
11. Remove PPE. Wash hands before leaving the laboratory or starting another activity

After incubation, plates can be stored wrapped in plastic in the refrigerator, upside down for up to 1 week.

Plate examination (NB. The plate must remain sealed during investigation).

1. Examine the plates for a lawn growth and any signs of contamination. Look for zones of clearing around the discs. Figure 1 shows a lawn culture of *E. coli* with discs containing different antimicrobial agents grown on a nutrient agar plate for 24-48 hours at a maximum of 30˚C.

Figure 1. A lawn culture of *Escherichia coli* with discs containing different antimicrobial agents grown on a nutrient agar plate for 24-48 hours at 30˚C.

2. Results may show:
   - Microbial growth up to the disc, with no zone of clearing. This is recorded as **resistant** to the antimicrobial agent,
     or
   - Zones of clearing around the discs recorded as **sensitive** to the antimicrobial agent.

3. Measure the diameter of any zones of inhibition including the diameter of the disc with a millimetre ruler, see Figure 2. Record the results in a table. The bigger the zone of inhibition, the more sensitive the bacteria are to the antimicrobial agent.

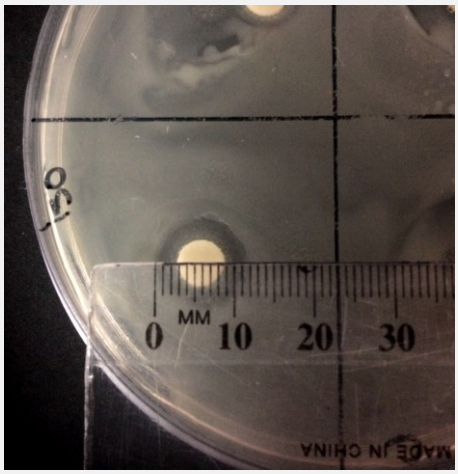

Figure 2. Measuring the zone of inhibition of isopropyl alcohol on *Escherichia coli* grown on a nutrient agar plate

4. After plates have been examined, autoclave or sterilise at 121ºC, 15psi for 15-30 minutes before double bagging and disposing in the normal waste bin.
5. Decontaminate the work bench with 70% v/v ethanol.
6. Remove PPE and wash hands.

7. Trouble shooting/emergencies

- **First Aid:** See latest SDS for ethanol, antiseptics or disinfectants used for more detailed information
  - **If swallowed:** Do not induce vomiting. Rinse mouth with water, and then give water to drink. Seek medical attention.
  - **If in eyes:** Hold open and irrigate with copious quantity of water. Seek medical attention.
  - **If on skin or hair:** Flush skin and hair with running water (and soap if available). Seek medical attention if irritation occurs.
  - **For further advice** contact the Poisons Information Centre on 131126.
- **No lawn culture produced and single colonies evident**:
  - Insufficient culture picked up with the swab and placed onto the surface of the agar, or concentration of organisms in the broth is too low
- **Different shapes, sizes and coloured colonies growing on the surface of the agar plate:** indicates contaminating organisms. This may have resulted from:
  - Nutrient agar not sterile
  - Broth culture containing the RG1 microorganism for lawn culture preparation was contaminated.
  - Breach of aseptic technique whilst preparing the lawn culture and/or placing on the paper discs containing antimicrobial agents.
- **Discs moving across the surface of the agar plate:**
  - Use capillary action to wet each disc to avoid oversaturation of the discs.

8. Waste disposal

- Contaminated forceps should be placed into a container such as a beaker for sterilising in an autoclave or pressure cooker.
- Paper towel should be placed into an autoclave or oven bag and sterilised at 15psi, 121˚C for 15–30 minutes, before being disposed of in the regular waste.
- Culture media containing microbes such as broth or agar plates should be autoclaved in their original containers prior to disposing in the regular waste. It is very important that bottles with lids should be loosened before putting through an autoclave or pressure cooker.
- Do not autoclave paper towel of any item that has been in contact with ethanol. These can be placed into the general waste bin.

9. Related material

- Risk Assessment
- Safety data sheets for microorganisms, antiseptics and disinfectants used, and nutrient agar
- See the following Science ASSIST material on:
  - SOP: Operating a pressure cooker and autoclave
  - SOP: Preparing nutrient and plain agar plates
  - SOP: Preparing a bacterial lawn culture

References and further reading

Course Hero. *'Microbiology – Testing the Effectiveness of Antiseptics and Disinfectants'*, Retrieved from Course Hero website, https://www.coursehero.com/study-guides/microbiology/testing-the-effectiveness-of-antiseptics-and-disinfectants/ (Accessed January 2023)

Hudzicki, J. 2009. 'Kirby-Bauer Disk Diffusion Susceptibility Test Protocol', Retrieved from The American Society for Microbiology website, https://asm.org/Protocols/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Pro (Accessed December 2021)

Royal Society of Biology. '*Aseptic Techniques'*, Royal Society of Biology website, https://practicalbiology.org/standard-techniques/aseptic-techniques (Accessed January 2023)

Royal Society of Biology. '*Making a spread or 'lawn' plates'*, Royal Society of Biology website. http://www.nuffieldfoundation.org/practical-biology/making-spread-or-%E2%80%98lawn%E2%80%99-plate (Accessed January 2023)

Microbiology Society. 2016. *Practical Microbiology for Secondary Schools.* Microbiology Society website, https://microbiologysociety.org/publication/education-outreach-resources/practical-microbiology-for-secondary-schools.html

Science ASSIST. 2023. *Standard Operating Procedure for Operating a pressure cooker and autoclave.* Retrieved from the Science ASSIST website, https://assist.asta.edu.au/resource/2924/sop-operating-pressure-cooker-and-autoclave

Science ASSIST. 2023. *Standard Operating Procedure for Preparing nutrient and plain agar plates.* Retrieved from the Science ASSIST website, https://assist.asta.edu.au/resource/4755/microbiology-sops-updated-school-level-2

Science ASSIST. 2023. *Standard Operating Procedure for Preparing a bacterial lawn culture.* Retrieved from the Science ASSIST website, https://assist.asta.edu.au/resource/4756/microbiology-sops-updated-school-level-3

Southern Biological. *'Exploring Antibiotic Resistance PDF'*, Retrieved from Southern Biological website, https://www.southernbiological.com/exploring-antibiotic-resistance-pdf/ (Accessed January 2023)