Bio Factsheet

Number 71

The Control of Bacteria

Most current A-level Biology syllabuses require candidates to study microbiology, biotechnology and health. Thus it is important for candidates to understand the need for the control of microorganisms, and to practice good technique if they perform practical work.

Controlling bacterial populations is important in several different respects to **reduce unwanted bacterial contamination** and to **minimise the chances of infection**. Control measures are used for:

- the removal of bacteria from hospital equipment, walls, floors and operating theatres.
- the control of bacteria in laboratories, experiments, industrial processes such as fermentations and in working environments in general.
- the reduction of the bacterial population in the home.
- the removal of unwanted bacteria from food and drink and proper food hygiene when preparing food.
- the treatment of bacterial disease and the prevention of disease spread through populations.

It is also necessary to control other types of disease-causing organisms such as viruses. The control measures that work on bacteria will also work on viruses, with the exception that antibiotics do not work on viruses.

Control measures which can be used include:

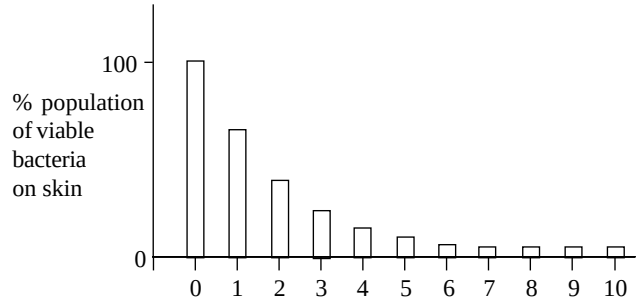
- general washing and personal hygiene.
- the use of chemical disinfectants.
- the use of dry heat.
- the use of moist heat and the autoclave.
- sterilisation by filtration or by the use of radiation.
- the use of aseptic technique in microbiological work.
- the use of antibiotics to treat or prevent disease.
- the use of vaccination programmes to reduce the risk of epidemics or pandemics and of individuals contracting dangerous illnesses.

An epidemic is when a disease occurs significantly above the expected level among the individuals in a population. A pandemic is when a disease spreads through populations in many countries or on a world-wide scale.

Disinfection by general washing and personal hygiene

The effectiveness of regular washing and scrubbing, using a good quality soap is illustrated by the graph in fig 1.

Fig 1. The effect of regular efficient hand washing on the bacterial population of the skin.



Thus surgeons and nurses have a very efficient washing and scrubbing up procedure to follow before entering the operating theatre. Workers in the food industry should frequently wash their hands and always before handling food. Regular hand washing and general washing is also important in the home environment in order to keep bacterial contamination down. From the safety aspect in microbiology practical work, always **wash your hands frequently and efficiently and always just before you leave the microbiology laboratory.**

Some definitions

Disinfection is the freeing of an article from some or all of its burden of microorganisms. It will only kill or remove a proportion of microorganisms and is not effective against bacterial spores. When disinfection is used, if correctly carried out, it should render the article safe. Disinfection may involve cleaning and scrubbing, the use of hot or boiling water, the use of ultraviolet light, or the use of chemical agents.

Sterilisation is absolute in that it kills all microorganisms including resistant spores. It could involve the use of the hot air oven to give dry heat, filtration methods, superheated steam under pressure in the autoclave, or the use of gamma-radiation for large scale industrial sterilisations (such as the bulk sterilisation of plastic syringes).

Bacteriostatic means that the disinfectant (or antibiotic) prevents the bacteria from reproducing but does not actually kill them.

Bacteriocidal means that the disinfectant (or antibiotic) or sterilisation technique kills the bacteria.

Antibiotic is a chemical produced by microorganisms, that, when used in dilute solution, can inhibit the growth of or kill bacteria (or fungi). Thus they are mainly used to treat bacterial diseases.

Vaccination (immunisation) is the process of making members of a population immune to a particular disease-causing organism. It involves injecting harmless forms of the disease-causing organism into people so that their immune response to the organism is established.

The use of chemical disinfectants

The disinfectant should be used '**concentrated enough for long enough to lower the number and types of microorganism present to a safe level**'. Remember that disinfectants will not kill all the spores, such as those of Clostridium sps. (tetanus, botulism, gas gangrene) and Bacillus sps. (anthrax, dysentery), so that if pathogenic sporulating organisms are present then sterilisation methods must be used. Disinfectants work better under warm conditions than cold, work best at particular pHs, are often inactivated by organic matter (e.g. faeces), work less well in hard water and some are inactivated by soap. They may be narrow spectrum, acting against a few types of organism only, or wide spectrum, acting against a wide variety of microorganisms. Table 1 shows the characteristics of some popular disinfectants.

Table 1. Characteristics of popular disinfectants

Class	Examples	Activity	Inactivated by	Comments
Phenols and cresols	Jeyes fluid, Izal, Dettol, Clearsol, Hycolin, Stericol, Sudol, Hexachlorophene	Wide spectrum against Gram +ve bacteria. Little effect on Gram –ve bacteria such as Pseudomonas.	Dettol is inactivated by organic matter but the others are not, but they are inactivated by plastic mop heads.	Hexachlorophene is incorporated into soaps and hand creams but the others are skin irritants. Good for disinfecting toilets and bed pans but are strong smelling. Effectiveness greatly reduced by dilution.
Alcohols	Ethanol, Methanol	Wide spectrum and dissolve away fat deposits.	Poor penetration of organic matter	Coagulates protein so instruments should be cleaned before alcohol treatment. Useful for wiping surfaces and skin. Used as a solvent for other disinfectants.
Halogens	Chloros, Domestos, Milton, Iodoform	Wide spectrum but not effective against tuberculosis bacilli.	Chlorine type are inactivated by organic matter.	Have little wetting ability and so must contain a detergent to be active. May corrode metals, except stainless steel.
Aldehydes	Formaldehyde Gluteraldehyde	Wide spectrum and kills some spores	Poor penetration of fabrics and organic matter.	Useful for disinfecting clean instruments which may be damaged by heat. Irritating to living tissues.
Diguanides	Chlorhexidine (Hibitane)	Limited spectrum, poor against Gram –ve bacteria.	Inactivated by organic matter, cork, soaps, plastics and mop heads.	Useful as a skin disinfectant.
Quaternary ammonium compounds	Benzalconium chloride, Cetrimide	Narrow spectrum, bacteriostatic not bacteriocidal.	Inactivated by organic matter and soaps	Useful as a skin disinfectant. May allow Pseudomonas populations to flourish.

***Remember** – it is not necessary to learn all these disinfectants – you only need to know the broad principles of disinfection. The data in the table may be helpful to you when planning practical work in microbiology.*

Other methods of disinfection

Washing and rinsing utensils and clothing or bedding in hot water at 70 – 80°C for several minutes will kill most non-sporing pathogens. Syringes and instruments should be boiled in water held at 100°C, or kept in steam held at 100°C for a minimum of 5 minutes, to kill most non-sporing pathogens.

Ultraviolet light at wavelengths less than 330 nm is an effective bacteriostatic agent, producing thymine dimers in DNA and thus interfering with replication. Sunlight has some disinfecting ability since it contains UV light, but only down to a wavelength of 290 nm Work surfaces can be disinfected by irradiating with UV light from a mercury vapour lamp (240 –290 nm), but goggles must be worn to avoid the risk of conjunctivitis. There is a risk that the thymine dimers will dissociate in visible light so that the bacteria which were apparently dead become photoreactivated.

Pasteurisation is used to render milk safe. The milk can be held at 63 - 66°C for 30 minutes (holding method) or at 72°C for 20 seconds (flash method). This will kill all non-sporing pathogenic organisms, such as Mycobacterium tuberculosis, M. bovis, Brucella abortus and Salmonella sps. It will not kill all of the souring bacteria such as Lactobacilli, but these are not dangerous to health.

Methods of sterilisation

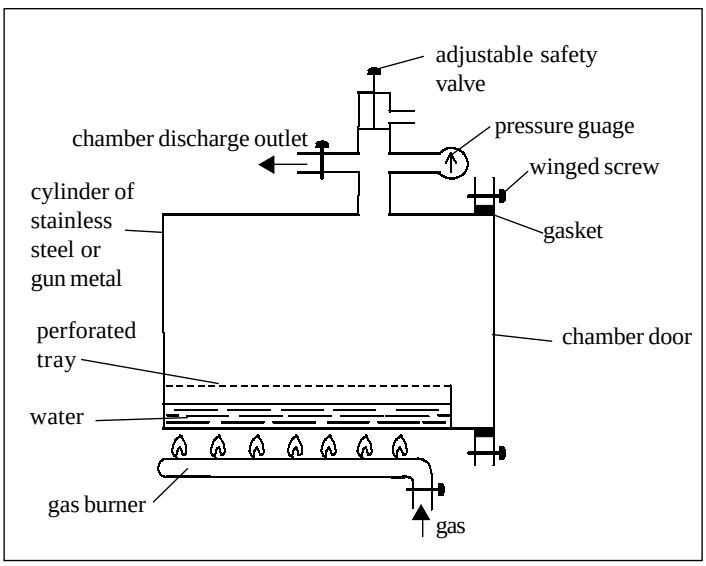
1. Dry heat

- Red heat can be used to sterilise inoculating loops, metal forceps or spatulas in the flame of a Bunsen burner. Alcohol flaming – when the object is dipped in alcohol and then the alcohol burned off, does not achieve a high enough temperature for sterilisation.
- The hot air oven, thermostatically controlled at 160°C for 1 hour, (not including warming up time), can be used for dry glassware, forceps, scissors, scalpels, swabs and syringes. Fats, oils and powders can also be sterilised in this way, providing they are placed in sealed containers.

2. Moist heat

- The autoclave sterilises using superheated steam under pressure. The articles being sterilised should be held at 121°C at 15 psi (pounds per square inch) for 18 minutes. (This does not include heating up time). Fig 2 shows the structure of a simple 'pressure cooker' type of autoclave.

Fig 2. 'Pressure cooker' type of autoclave



- Filtration can be used to sterilise fluids, such as liquid growth media. Bacterial filters generally have pore sizes less than 0.75 mm.
- Ionising radiation, such as X-rays or gamma rays, can be used for large scale industrial sterilisations, for example, sterilisation of plastic disposable syringes.

Exam Hint - *Exam Boards are unlikely to ask questions about the different techniques of disinfection and sterilisation, but the details given here may help you when planning and performing microbiological practical and project work. Exam Boards are likely to ask candidates questions about antibiotics and vaccination.*

Antibiotics and their use

An antibiotic is a chemical produced by a microorganism which inhibits the growth of other microorganisms such as bacteria or fungi. An antibiotic that is effective against a wide range of microorganisms is called a **broad spectrum** antibiotic. An antibiotic which only affects a few species is called a **narrow spectrum** antibiotic. Tetracyclines and chloramphenicol are broad spectrum antibiotics but penicillin and griseofulvin (anti-fungal) are narrow spectrum. Antibiotic resistant strains of bacteria do not respond to treatment with antibiotics and so are proving a problem in disease control and treatment. The number and variety of resistant strains of bacteria are continuing to increase but no significantly new antibiotics are available. Research is directed at modifying the molecular structures of existing antibiotics rather than discovering new antibacterial substances which might act in a different way. Table 2 names some common antibiotics and gives details of their actions and uses.

Vaccination

About 60 vaccines are in common use across the world, enabling some protection against not only bacterial diseases, but also against viral, protozoal and helminthic (worm) diseases. Vaccines are made from live attenuated (weakened by heat or chemical treatment) strains of the organism, from dead organisms, from parts of organisms and from modified toxins produced by the organisms. All of these contain antigens and so cause an immune response to be established. The attenuated strains do not cause as severe an infection as the normal microorganisms but do cause adequate immunological protection to be developed. This immunity is long lasting, **active immunity**, because B and possibly T memory cells are produced and stored.

The vaccine may be given to the patient, usually a child, by injection into the skin layers, muscle or bloodstream, or via the mouth if an oral vaccine. The antigenic components of an oral vaccine can pass through the stomach and be absorbed through the gut wall without damage.

Several factors influence the effectiveness of a vaccine:

- the size of the immune protection (indicated by the level of antibody produced in the blood) may be increased by giving booster doses of the vaccine. The timing and number of the booster doses varies according to the vaccine involved. For instance, booster doses of anti-tetanus vaccine should be given every five years to maintain full protection. Three spaced doses of DPT vaccine (diphtheria, pertussis, tetanus) are given to young children, starting when the child is four to six months old.
- many pathogenic bacteria contain several different strains of the species. The vaccine needs to contain antigens from all the strains to be useful.
- certain chemicals, known as adjuvants, may be added to the vaccine to make weak antigens produce a stronger immune response.

Effective widespread vaccination not only protects the individual but reduces the frequency of disease in the population since it results in a smaller reservoir of the pathogenic organism in the population and so non-vaccinated individuals are less likely to come into contact with the pathogen. Sometimes actual antibodies are injected into patients to give short term **passive immunity**. An example is the injection of tetanus antibodies into a person who may have been infected with the bacteria. The antibodies give protection until the active immune response develops.

Table 2. Some antibiotics and their actions and uses

Antibiotic	Activity/use
Penicillin	Blocks peptidoglycan synthesis in cell wall synthesis in Gram positive bacteria. Active against Pneumococcus (pneumonia), Clostridium (tetanus), Corynebacterium (diphtheria), Streptococcus (scarlet fever) and Treponema (syphilis).
Chloramphenicol	Blocks peptide bond synthesis so inhibits polypeptide synthesis and growth. Active against Salmonella (typhoid), Staphylococcus (skin infections), Haemophilus (meningitis).
Streptomycin	Inhibits correct amino acid incorporation into polypeptides and thus inhibits growth. Active against Mycobacterium (tuberculosis).
Tetracycline	Prevents tRNA from binding to prokaryotic ribosomes and so inhibits polypeptide synthesis and growth. Broad spectrum antibiotic.
Erythromycin	Inhibits prokaryotic ribosomes and thus stops polypeptide synthesis and growth.
Rifampicin	Inactivates bacterial mRNA polymerase thus preventing transcription and bacterial development.

Practice Questions

1. Tuberculosis is caused by a bacterium which lives in the lungs and causes destruction of tissue. Tuberculosis bacteria exposed to the air form resistant spores. Overcrowded living conditions encourage the spread of tuberculosis.

(a) (i) Describe how tuberculosis may spread from person to person. 2

(ii) Explain why tuberculosis spreads more easily in overcrowded living conditions. 1

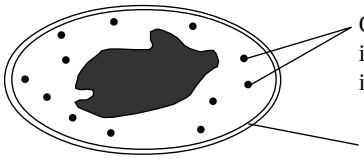
(iii) During the 1950s and 1960s when tuberculosis was very common in the United Kingdom, buses and railway carriages often had a notice saying 'no spitting'. Explain why. 2

(b) Vaccination is used to control the spread of tuberculosis. Give <u>two</u> reasons why children should be vaccinated, even though tuberculosis is now quite rare in the United Kingdom. 2

(c) People who develop tuberculosis are treated with antibiotics. Explain why. 2

Total 9

2. The diagram shows the cell of a bacterium. The site of action of two antibiotics is labelled on the diagram.



(a) Explain why:

(i) penicillin is safe to use in the human body to treat diseases caused by bacteria. 1

(ii) chloramphenicol may have side effects affecting bone marrow. 2

(b) (i) Distinguish between 'broad spectrum' and 'narrow spectrum' antibiotics. 1

(ii) suggest why penicillin is classed as a narrow spectrum antibiotic but tetracycline is classed as a broad spectrum antibiotic. 4

Total 8

3. Immunity can be defined as the ability to resist infection.

(a) A person can acquire immunity actively or passively. Complete the table to show some of the features of passive and active immunity. Place a tick in the box if the feature applies and a cross if it does not apply.

	the person produces an immune response	the person produces memory cells	the immunity can be acquired naturally and artificially
passive immunity			
active immunity			

2

(b) Some vaccinations contain attenuated microorganisms, others contain killed microorganisms. It is now thought preferable to develop vaccines containing attenuated microorganisms wherever possible.

(i) What is an attenuated microorganism? 2

(ii) Suggest why vaccines containing attenuated microorganisms might be preferred to those containing killed microorganisms. 2

Total 6

Answers

1. (a) (i) exhaled air contains droplets of water containing the bacteria; infected droplets inhaled by another person; 2

(ii) greater risk of inhaling infected droplets; 1

(iii) spit contains bacteria which would form spores; spores would blow into air/be on surfaces and so could infect people; 2

(b) may visit a country where TB is common;
ref to 'herd effect'/large number vaccinated gives protection to whole population;
TB is becoming harder to treat since antibiotic resistant strains have developed; max 2

(c) antibiotics specifically attack/kill bacteria;
ref to use of Streptomycin; 2

Total 9

2. (a) (i) only affects cell walls which are not present in human cells; 1

(ii) inhibits protein synthesis in human cells;
bone marrow cells constantly growing/dividing so have a high level of protein synthesis/ may inhibit blood cell formation; 2

(b) (i) broad spectrum antibiotics are effective against a wide range of microorganisms but narrow spectrum antibiotics only act against a few species; 1

(ii) penicillin blocks peptidoglycan synthesis in cell walls (making bacteria burst due to weakened cell walls);
peptidoglycans occur only (in large amounts) in Gram positive bacteria;
tetracycline inhibits tRNA attachment to prokaryotic ribosomes preventing protein synthesis/growth;
thus tetracycline will be indiscriminate in the organisms they inhibit/kill; 4

Total 8

3.(a)

	the person produces an immune response	the person produces memory cells	the immunity can be acquired naturally and artificially
passive immunity	✗	✗	✓
active immunity	✓	✓	✓

(b) (i) living but modified organism;
given heat/chemical treatment;
has reduced reproduction rate/infectivity; max 2

(ii) more closely resembles a real infection;
provokes a better immune response/killed organisms may have modified antigens;
smaller inoculum required/immunity may be longer lasting; max 2

Total 6

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ISSN 1351-5136