Answers to self-assessment questions

CHAPTER 1

1. The origins and modes of transmission of microbial infection were poorly understood principally because they could not be seen. The gradual development of better microscopy techniques provided proof that microscopic living organisms could be the cause of illness. As early as the sixteenth century it had been recognized that disease could pass from one person to another; this had been observed in sexually transmitted disease. At the end of the seventeenth century the discovery was made that if flies were not allowed near meat, no maggots appeared; a century later Pasteur demonstrated that microorganisms were carried in the air. The ability to grow bacteria on solid culture medium was another milestone along with others at the end of the nineteenth century, culminating in Pasteur’s germ theory of disease and Koch’s postulates.

2. There are several important ways in which prokaryotic and eukaryotic cells differ. Prokaryotes are smaller (less than 5 µm) and single cells, whereas eukaryotic cells are greater than 10 µm and often multicellular. Prokaryotic DNA is super-coiled, not enclosed in a membrane-bound organelle. In fact they do not have organelles and their DNA is not associated with histones or proteins. Eukaryotes have membrane-bound nuclei and organelles, and linear DNA with a high histone content; they associate with non-histone proteins to form chromatin. Prokaryotes have no cytoskeleton and complex cell walls, while eukaryotes always have cytoskeletons and simple cell walls. Whereas prokaryotes grow by asexual binary fission, eukaryotes can divide by mitosis or meiosis and reproduction can be sexual or asexual. Their ribosomes are of different sizes: prokaryotic ribosomes are 70S and eukaryotes have ribosomes of 80S. Eukaryotes use common metabolic pathways; prokaryotes use a variety. The ability to form biofilms is a feature of prokaryotes. Both prokaryotes and eukaryotes have motile genera; prokaryotes via flagellae composed of flagellin, eukaryotes by flexible waving cilia or flagellae composed of tubulin.

3. The species affecting the blood could be *sanguis* or *sanguinis*. The enteric species could be *enterica*, *colonica*, *jejuni*, *duodenale*, etc. The species infecting the skin could be *epidermidis* or *epiderma*.

4. Simple phenotypic tests for the identification of an unknown bacterium could include a Gram stain which would give an indication of Gram reaction, shape and size. The atmosphere and temperature at which it grew, observation of any change to the selective culture medium and some simple biochemical tests would provide further evidence.
5. A species of bacteria is a population of cells that share similar characteristics within a genus. One species may be harmful to humans and cause serious disease, whereas a different species may be harmless. An example of this is to be found in the genus *Streptococcus*. *Streptococcus pyogenes* can cause sore throats and serious systemic disease. *Streptococcus oralis* is a member of the normal flora of the mouth and is rarely involved in disease. Knowledge of a bacterial species has a practical use in identification and diagnosis of disease. A strain is a subset of a bacterial species descended from a single organism. Clinical isolates, for example, may be of the same species but are different strains; they often have slight differences in virulence, biochemical reactions and/or antibiotic susceptibility. A collection of strains that share certain properties can be called the same species even if they differ by up to 30% in DNA variation.

CHAPTER 2

1. These three types of bacterial cell walls all possess an inner cytoplasmic membrane and all contain peptidoglycan and membrane proteins. Gram-positive bacteria have a thicker peptidoglycan layer that provides a rigid shape for the cell. Lipoteichoic acid and teichoic acid are also present. Gram-negative cell walls have two membranes; the outer membrane, called endotoxin, is specialized and consists of lipid A, core oligosaccharides and linear polysaccharide. In addition, Gram-negative cell walls have a periplasmic space containing enzymes for degrading macromolecules and antimicrobial substances. Acid-fast cell walls are classed as Gram-positive but also contain lipids which render them resistant to staining and disinfectants. The nature of the cell wall also makes them less permeable to nutrients and consequently slower-growing and more resistant to antibiotics. There are several ways in which the structure of the cell wall affects bacterial behaviour, and any two examples from the following are appropriate answers: Gram-positive bacteria are able to survive in drier environments, yet remain porous enough to absorb nutrients. Some of their cell wall structures are used for attachment to host cells. Gram-negative bacteria survive well in moist environments. Their LPS can provide antigenic diversity (there are over 2000 different serovars of *Salmonellae*) as well as having a toxic effect upon the host when the lipid A is released at cell death. They are more resistant to antibiotics because enzymes in the periplasmic space can destroy many antibiotics and porin proteins in the outer membrane have greater permeability for hydrophilic molecules. Acid-fast bacteria grow more slowly than Gram-positive or -negative cells because they are less permeable to the transport of nutrients into the cell. They are also more resistant to antibiotics.

2. A motile bacterium is able to move around in the environment or within a host by means of flagellae, composed of the protein flagellin. These are long thin appendages arising from the cytoplasmic membrane and extending through the cell wall into the surrounding medium. Flagellae consist of a basal body, a hook and a filament composed of the protein flagellin. Power to drive the flagellae is derived from proton motive force across the membrane, which generates ATP, which in turn provides the energy. Flagellae can be positioned all over the bacterium, at one end or at both ends. Movement is achieved by a series of runs and tumbles and allows bacteria to move towards a nutrient source and move away from a repellent substance. Flagellae can
also have a role in the infectious process by allowing bacteria to penetrate mucous layers above the epithelium and reach their receptor sites.

3. The genera *Clostridia* (anaerobic bacteria) and *Bacillus* (aerobic bacteria) both produce spores and between them account for several serious diseases, e.g. anthrax, tetanus, botulism, gas gangrene and antibiotic-associated diarrhoea. When the bacteria are stressed for nutrients they transfer all their genetic material into spores which form within the cells before death. The spores have a hard outer coat and can therefore survive in the environment for indeterminate periods of time. They come into contact with new hosts when they are transmitted either by dust particles in the air (a significant problem, for example, with *Clostridium difficile* in healthcare settings), contaminated soil entering a wound or via food. Once in the nutrient-rich, warm body of a host they vegetate and become viable bacteria and as they grow, they emit powerful toxins characteristic of the diseases they cause. Because of their tough outer spore coat they are a challenge for disinfectant control and for sterilization procedures, which have to demonstrate the ability to kill spores.

4. Strict or obligate aerobes can only grow in the presence of oxygen. Facultative anaerobes, as the name suggests, are able to grow in the presence or absence of oxygen. Aerobic respiration is more efficient and so facultative anaerobes grow better in oxygen. Aerotolerant anaerobes can grow in the presence of oxygen, but do not use it in metabolism. Micro-aerophilic bacteria are aerobic but require lower oxygen levels than air, for example *Campylobacter* species are grown on specialized culture media in artificially lowered levels of oxygen. Capnophilic bacteria prefer an environment where the carbon dioxide is higher than normal. This enhanced growth is a feature of *Neisseria gonorrhoeae*, *N. meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae*. Obligate anaerobes are killed by the presence of oxygen, so if their presence is suspected in a clinical sample they must be cultured on appropriate media and incubated in anaerobic conditions immediately. Strictly aerobic bacteria are not found in deeper areas of the body where oxygen is limited. Micrococci, for instance, are Gram-positive cocci found on the skin but not in deeper tissue. Facultative anaerobic bacteria such as *Escherichia coli* can grow in a wide range of body sites – they thrive on moist areas of the skin with abundant oxygen and also within the colon. Some members of the genus *Clostridia*, including *C. histolyticum*, are aerotolerant anaerobes and although they prefer anaerobic conditions, they will survive in the presence of oxygen. Micro-aerophilic bacteria such as *Campylobacter* species cause infection in the intestines; they can also survive in the blood. Strict anaerobes are found in deeper sites of the body. *Clostridium perfringens* causes infections within tissue and can also create its own anaerobic environment by breaking down tissue by excreting protein toxins, leading to gangrene.

5. Selective toxicity describes the process where pathogenic cells are targeted by antimicrobial drugs, with the drugs targeting structures that are different from human cells. This minimizes the destruction of host cells in the treatment process. Bacteria are prokaryotes and therefore have different cell walls and membranes, different sizes of ribosomes and different protein synthesis and genetic replication strategies, all providing effective drug targets. Fungi and protozoa are eukaryotes, with more similarities with human host cells. Fungi are also slower growing, necessitating longer treatment with fewer drug options. Protozoa can have complex life cycles which also
Answers to self-assessment questions need to be understood in terms of control and treatment. Part of the complex life cycle can also involve periods of latency, such as *Toxoplasma gondii* show in tissues, making treatment difficult and also providing the potential for reactivation if the patient becomes immunocompromised. Treatment of protozoal cysts is also more difficult to achieve.

6. The four principal stages of the bacterial growth curve, during which bacteria undergo continued rounds of cell replication by binary fission, include the lag phase, log phase, stationary phase and decline or death phase. Not all bacteria will demonstrate a lag phase, where bacteria adjust to their new environment and synthesize cell constituents and enzymes and recover from any stress experienced in their previous environment. The logarithmic, exponential phase is the period when bacteria are replicating at their optimum, maximum rate. Growth is constant and if the log of the number of bacteria is plotted against time, the result will be linear. In a typical culture grown from a clinical specimen, the time for visible colonies (the result of exponential growth of one bacterium) to be seen is after 18–24 hours. Cultures of anaerobic bacteria may take at least 48 hours to produce visible colonies. On culture media there will come a point where the majority of the essential nutrients have been used up and there is a build-up of waste products. The growth rate slows and the curve levels off into the stationary phase. During this phase the number of cells dying is roughly equal to the production of new cells. The death rate increases in the last phase of the growth curve and is again exponential. The growth of potential pathogens in food samples can be controlled by creating conditions that are sufficiently inhibitory to prevent the bacteria entering the exponential stage. This can be achieved by manipulating the temperature, pH, moisture and salt content.

7. Opportunistic infections occur in patients who are immuno-compromised in some way, either because of medical treatment (chemotherapy, radiotherapy, invasive procedures), pre-existing clinical conditions or immune deficiency, for example as a result of infection with HIV. *Aspergillus* infections are a good example, targeting patients with cystic fibrosis or undergoing chemotherapy for leukaemia. *Candida albicans* is present in small numbers as part of the normal flora but can overgrow if given the opportunity when host defences are compromised. This can range from symptoms of oral thrush to severe systemic infections.

8. The infection cycle involves a definitive host, the cat, and the secondary hosts are grazing animals and humans. Humans come into contact with the oocysts excreted by cats, either in the soil or in food. Oocysts are consumed by grazing animals and can be passed to humans when they eat the meat, either undercooked or raw. Different preferences for cooking meat may account for the higher levels of toxoplasmosis in France, for example, where meat is more likely to be eaten raw or undercooked. Within the definitive feline host, trophozoites become merozoites and these differentiate into male and female gametocytes and are excreted in the faeces as oocysts. Oocysts mature in the environment and remain stable in the soil for months. Within the human host, the oocysts change into sporozoites which then invade tissues and form pseudocysts. They are controlled by the immune system, but if the immune system is compromised, as in AIDS patients, the disease can be reactivated. Infection during pregnancy is also a problem as the sporozoites can cross the placenta and damage the fetus. Serology tests, which look for the antibodies
formed to the infection, can distinguish whether the infection is newly acquired or a past infection controlled by the immune system.

9. The three classes of helminths are the nematodes, cestodes and trematodes. Nematodes are roundworms that inhabit either the intestines, blood or tissues. Eggs are passed from the host into the environment and thence into a new host, either by ingestion with food or water, or by skin penetration. The larvae of tissue and blood nematodes such as *Onchocerca volvulus* and *Wuchereria bancrofti* can be spread by flies and mosquitoes. Cestodes are tapeworms inhabiting the intestines of their host. Human infections arise from ingestion of tapeworm larvae in animal tissue, pork, beef or fish. The larvae then mature into segmented tapeworms and numerous eggs are shed as the segments are passed out in the faeces. The dog tapeworm does not form worms in humans, but if ingested by herbivores or humans, hydatid cysts are formed in the tissues. The trematodes are the flukes which infect human tissue and blood vessels. They use snails as intermediate hosts.

10. Climate, lifestyle, poverty and politics all influence the level of parasitic burden of the population. Warm moist environments provide ideal conditions for eggs to remain in the soil. In some countries public health and sanitation systems are not sufficiently developed to guarantee clean water for drinking or cooking, and this provides reservoirs for parasitic eggs. Lakes inhabited by snails provide intermediate hosts for trematodes shed from human sources. Stagnant water provides a breeding ground for flies and mosquitoes. Poor quality beef and pork may harbour tapeworm larvae. Appropriate treatment is often not available and the population harbour multiple parasitic infections. Education and eradication programmes require political will, financial and human resources, which are not always available. For example, schistosomiasis spread by snails could be eliminated by education about sanitation, elimination of the snails, ensuring water for human consumption is piped in snail-free pipes and making appropriate drug treatments available to all.

**CHAPTER 3**

1. The genomic structure of viruses comprises either DNA or RNA, but never both. The genomes can be double- or single-stranded (abbreviated to dsDNA, dsRNA, ssDNA or ssRNA). If the virus has a single RNA strand, it is classified as positive or negative, depending on whether the messenger RNA (mRNA) is derived directly from the genome (+) or from the complementary strand (-). The Baltimore classification includes these five classes plus reverse RNA (for example HIV) and reverse DNA (for example hepatitis B virus).

2. Viruses can be spread by the same routes of transmission as bacteria: respiratory, skin shedding, faecal–oral, sexual, blood, from animals and insect vectors and by inanimate objects (fomites).

3. Viral envelopes are lipid bilayers with proteins inserted in them. The lipid bilayer is often added to the nucleocapsid as the viruses leave the host cell, and consists of host cell proteins. The proteins are either matrix proteins that link the nucleocapsid to the envelope, or glycoproteins pointing out of the virus structure as spikes. These spikes
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can be used to attach to host cells, for example the H and N of influenza virus, and also give the virus a characteristic antigenic structure; again, influenza virus is a good example – H1N1, etc.

4. Electron microscopy, cell culture, molecular methods using PCR and enzyme-linked immunosorbent assays (ELISA) are most commonly used. ELISA is widely used, either manually or in an automated system, to diagnose hepatitis A and B, rubella, EBV, VZV, rotavirus, CMV and HIV, among others. Real-time PCR methods are used to diagnose influenza, HSV and hepatitis C, and to confirm HIV. Electron microscopy is useful in the diagnosis of gastrointestinal viruses such as adenovirus. CMV and HSV are sometimes diagnosed using cell culture. Cell culture methods are gradually being replaced by molecular methods.

5. HIV mutates very quickly and therefore if a single drug is used, resistance will develop very quickly. A combination of drugs is used, targeting different aspects of the viral replication cycle and monitored by measuring the viral load in the blood. The principal classes of drugs used are nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors, fusion and envelope inhibitors, integrase inhibitors and receptor antagonists.

6. The difference between an adapted virus and a re-assortment virus is that the adapted virus gradually changed its structure so that it was able to attach to human receptors and cause disease. The virus could then pass from human to human and infect large numbers of susceptible individuals. Re-assortment or recombination occurs when two viruses from different species mix within the same host and some of the RNA mixes, giving rise to a new genetic strain, which then goes on to infect large numbers of susceptible people.

7. Examples of a herpesvirus are HSV, EBV, HZV and CMV, and all of them cause a primary acute illness: cold sores, glandular fever, chickenpox and CMV respectively, and then become latent in nerve cells or blood cells, ready to reactivate if the immune system weakens. HSV can cause recurrent cold sores, Bell’s palsy and encephalitis; EBV can reactivate as glandular fever, cause post-viral fatigue and lead to lymphomas in the seriously immuno-compromised; HZV reactivates as shingles, while CMV causes pneumonia and retinitis in the seriously immuno-compromised.

8. It is important to ensure a laboratory diagnosis of infectious hepatitis because the causes, pathogenesis and outcomes are different, depending on whether the infection is caused by Hepatitis A, B or C. (Hepatitis E is unlikely to be a cause in the UK). Hepatitis A causes a self-limiting disease, resulting in clearance of the virus from the body and the formation of long-lasting antibodies. There is no long-term carriage. Hepatitis B is highly infectious, is an infection risk in the laboratory, and is transmitted through blood, needles and sexual transfer. The serology of the disease is complex – the patient may remain highly infectious and become chronically ill, with the possibility of developing cirrhosis or liver cancer in the long term. Hepatitis C carries similar risks of chronic disease.

9. Haemorrhagic fever viruses are transmitted by ticks, mosquitoes and via infected animals to humans. The specific vectors are not normally found in the UK. Visitors
to endemic areas can be vaccinated against yellow fever. Examples of VHF are Ebola virus, Marburg disease, Lassa fever, Rift Valley fever, dengue and yellow fever.

10. It is important to maintain high levels of immunity to mumps, measles and rubella and other childhood vaccine preventable disease in the community, in order to prevent circulation of the virus and disease incidence. If the number of susceptible individuals rises then the viruses are able to spread among communities. Falling levels of uptake of the MMR vaccine resulted in a mumps epidemic in 2005, with over 40 000 cases. Measles levels also begin to rise if MMR uptake is lower. Although childhood rubella infection is mild, the risk to pregnant women is high, because rubella infection can be passed across the placenta and cause serious congenital defects. Measles and mumps are also more serious if caught in adult life, and measles can also give rise to complications in childhood illness. If the level of these diseases has been low for many years there is also less awareness of the severity of the infections. Polio was eradicated in this country by vaccination and continues to be part of the UK vaccination programme, but has not yet been fully eradicated worldwide.

CHAPTER 4

1. An opportunistic infection is caused by a microbe which is usually considered to be of low pathogenicity in a healthy host, taking the ‘opportunity’ to gain access to tissue or multiply on a mucosal surface when the immune response of the host is compromised. Opportunistic pathogens can be members of the normal flora or can be acquired from the environment. Coagulase-negative staphylococci and *Candida albicans*, both members of the normal flora, are often implicated. Environmental bacteria such as *Pseudomonas aeruginosa* and the fungus *Aspergillus niger* are examples of environmental opportunists causing infections in the lungs of susceptible patients. The increased use of chemotherapeutic drugs, and the introduction of catheters and lines into patients have increased the number of immuno-compromised patients.

2. Two possible explanations for the patient’s diarrhoea are: a) Antibiotics are still active as they are passed out of the body through the intestinal tract, and as they come into contact with the normal gut bacteria they destroy the protection afforded by their presence. Diarrhoea can be a side-effect of a course of antibiotics. b) If the patient is in an environment where there are *Clostridium difficile* spores, these can infect the patient and occupy the niches left by the normal flora. This antibiotic diarrhoea can be self-limiting or it can progress to the more serious condition of pseudomembranous colitis, where a membrane of fibrin, white cells and mucus forms over the intestinal lumen. The symptoms are caused by the production of toxins A and B. The presence of the toxins can be detected from a faecal sample.

3. Members of the normal flora can lead to infection by gaining access to areas of the body outside their normal habitat. For example, gut and vaginal flora can lead to urinary tract infections if they are able to attach to the epithelial cells of the urethra and ascend into the bladder. Streptococcal bacteria from the mouth can travel in the bloodstream and lodge on damaged or prosthetic heart valves, causing bacterial endocarditis. Perforation of the intestine from trauma or during surgery can allow gut bacteria to spill out into the pelvic cavity and cause serious infections.
4. The following are components of the innate immune system: physical barriers, mechanical removal, changes in pH, antibacterial substances, pattern recognition receptors, neutrophils and complement.

5. The alternative pathway, where complement protein C3b is deposited on bacterial cell surfaces. The mannose-binding lectin pathway, where mannose on the surface of bacteria is bound by mannose-binding lectins (MBLs); mannose-binding lectin-associated serine protease then binds to the bacteria-bound MBL and activates complement protein C4. The classical pathway is initiated by complement protein C1qrs attaching to pre-formed antibody attached to the bacterial surface.

6. Foreign antigen is processed within the antigen-presenting cell and broken down into peptides. These peptides are then associated with the MHC antigens on the cell surface and presented to a small subset of lymphocytes, whose T cell receptor recognizes that particular MHC peptide combination and becomes an effector cell. Superantigen toxins bypass processing and presentation in association with MHC by binding to the side of the receptors and bringing the antigen-presenting cell and the T cell together in an unregulated way. This can result in either over- or under-stimulation of an appropriate response.

7. Any two of the following strategies: capsules provide protection because the complement protein cannot easily opsonize the bacterium and even if it were able to, the attack complex is sufficiently far away from the cell wall. C3b can be inactivated by bacterial proteases. M protein found on *Streptococcus pyogenes* binds complement regulatory proteins and prevents opsonization by C3b. Gram-negative bacteria with long LPS side chains prevent the membrane attack complex from damaging their cell walls because the C3b attaches at too great a distance from the wall.

8. Any of the following diseases: anthrax (*Bacillus anthracis*), botulism (*Clostridium botulinum*), *Clostridium difficile*-associated diarrhoea, gas gangrene (*Clostridium perfringens*), tetanus (*Clostridium tetani*), diphtheria (*Corynebacterium diphtheriae*), whooping cough (*Bordetella pertussis*), cholera (*Vibrio cholerae*). The three classes of toxins are membrane-acting toxins, A–B toxins and superantigen activity.

9. Two component regulatory systems consist of a sensor on the cytoplasmic membrane that responds to changes in temperature and the level of essential ions, such as magnesium, iron and phosphorus. Signals are sent from this protein to the regulator protein in the cytoplasm by a series of phosphorylation events. The regulator protein responds to the signal by binding to a specific area of DNA which controls a gene or series of genes. These genes are then switched on and the virulence factor expressed; perhaps a haemolysin to break down red cells and increase the iron levels. When the concentration again reaches a satisfactory level, the sensor no longer sends a signal to the regulator and the genes are switched off.

10. Direct contact (skin cells); respiratory (via droplet infection from coughing and sneezing); fomites (which could be clothing, doorknobs, etc.); faecal–oral transmission (via food, water and poor hygiene); sexual transmission; blood-borne (via contaminated needles, contact with blood from infectious individuals); via animals, vertebrates or vector-borne.
1. The development of the condenser, by Abbe, enabled light to be concentrated and focused onto the sample. Construction of the compound eyepiece and objective lenses reduced the distortion of the final image. The objective lens, which is nearest to the specimen, produces the initial magnification and the lens of the eyepiece further magnifies it. The production of glass of a higher quality also significantly improved the quality of the lenses.

2. The objective lenses give a choice of 10×, 40× and 100× magnifications. The magnification of the eyepiece, 10×, is then included, giving magnification values of 100×, 400× and 1000×.

3. The resolution of the light microscope is half the wavelength of the light used, which is 400 nm or 0.4 µm, giving a resolution of 0.2 µm. Most bacteria are of this approximate size and so they are able to be visualized by the light microscope. However, details such as flagellae cannot be seen and would need the higher resolution of the electron microscope (EM). The magnification achieved by EM is 250 000×, allowing the ultrastructure of bacteria and the structure of viruses to be seen.

4. Urine samples are pipetted into flat-bottomed microtitre trays and allowed to settle. The inverted microscope then gives an image of the cells that have settled onto the base of the well. An infected urine sample would contain significant numbers of white blood cells, and possibly red blood cells, bacteria and protein casts.

5. While some ova and cysts are unique in their structure and easily recognizable, others need to be measured before identification can be positively made. Examples of this are cysts such as *Entamoeba histolytica, Entamoeba coli, Blastocystis hominis* and *Endolimax nana* and the ova of *Hymenolepsis nana*.

6. Any four of the following: Urine and faecal samples, Gram stains, identification of *Trichomonas vaginalis*, examination of fungal cultures and the examination of cerebrospinal fluid.

7. Darkground illumination can be used to visualize *Treponema pallidum* spirochaetes in samples taken from the initial chancre, or ulcer, in a patient suffering from syphilis. The specimen does not need to be stained or processed in any way. Similarly this type of microscopy can be useful, although not reliable, for visualizing spirochaetes of *Leptospira* species in plasma or serum specimens.

8. FITC, green fluorescence, TRITC, phycoerythrin or Texas red for red fluorescence and DAPI and calcofluor for blue fluorescence. The difference between the direct and indirect methods is that in the direct method, antigens expressed in the specimen are visualized directly by the attachment of labelled specific antibodies, e.g. *Chlamydia trachomatis* elementary bodies in endocervical swabs, urine or eye swabs. The indirect method is used to demonstrate the presence of antibodies in patient serum samples by first incubating the serum with a substrate containing the antigen of interest. After washing away unbound antibody, the antigen–antibody complex is incubated with a preparation of fluorescently labelled second antibodies (often
anti-human IgG) which attach to the bound antibody. This is in order to visualize the antigen–antibody reaction. e.g. *Legionella pneumophila* antibodies in serum detected using *Legionella* bacteria as the substrate; and the binding of autoimmune antibodies in patient serum to tissue substrates such as mouse liver and kidney.

9. A much greater resolution and magnification can be achieved with EM and SEM, allowing more detail and smaller structures, e.g. virus particles, to be identified. Additionally, SEM uses video technology to reproduce the images and allows denser and larger surface structures to be seen. This has been useful in research to visualize infection processes.

10. The slide is first flooded with crystal violet for one minute and then washed in tap water. Gram’s iodine is then applied for one minute and the washing step repeated. Decolorization is achieved by the addition of acetone, only for about 10 seconds. If the slide is over-decolorized, Gram-positive bacteria may appear falsely negative. On the other hand, under-decolorization would suggest that Gram-negative bacteria are falsely identified as Gram-positive. The slide is washed after the acetone step and then counterstained with carbol fuchsin, safranin or neutral red for one minute, then washed and blotted dry.

**CHAPTER 6**

1. The use of aseptic technique: use a sterile loop, either a wire loop sterilized in a hot Bunsen flame, or a sterile disposable plastic one. Keep the bottle in a wire rack when not in use, to prevent spillage onto the bench. Hold the sterile loop in the right hand, the culture in the left hand and remove the lid of the bottle with the fourth and fifth fingers of the right hand. Remove a loopful of liquid culture and replace the lid carefully before returning the bottle to the rack. For cultures growing on an agar plate, remove a colony with the loop and only have the lid off the culture plate for as short a time as possible. Inoculate the plate and then resterilize the loop or dispose of a plastic one in the appropriate container. Any contamination of gloves or the bench should be dealt with immediately.

2. The viable count per ml can be calculated by the Miles and Misra method. A series of tenfold dilutions of the sample are prepared using distilled water, a balanced salt solution or nutrient broth as diluent. The method can be used simply to find out the cell count of the original culture or to demonstrate growth characteristics of bacteria on different culture media formulations. A known volume of each dilution is then added to a whole plate (typically 1 ml), or to a segment of a culture plate (typically 25 μl per segment of a well-dried plate is the maximum volume, to avoid mixing of dilutions). After incubation the whole plates, or segments, are observed and counts performed on areas where the colonies are separate and it is possible to count them. The number of colony-forming units (CFU) per ml (the viable count/ml) is calculated as follows:

\[
\text{number of colonies counted} \times \frac{1}{\text{vol}} \times \text{dilution} = \text{CFU/ml}
\]
Therefore if 6 colonies were counted on the $10^5$ segment, the calculation would be:

\[ 6 \times \frac{1}{0.025} \times 10^5 = 6 \times 40 \times 10^5 = 240 \times 10^5 = 2.4 \times 10^7 \text{ CFU/ml} \]

3. The catalase test will distinguish between two major groups of Gram-positive cocci, the staphylococci (catalase positive) and the streptococci (catalase negative). The coagulase test can then be used on the presumptive staphylococci to further distinguish between \textit{Staphylococcus aureus}, which is coagulate positive, and coagulate-negative staphylococci.

4. The basis of the streptococcal grouping test is that different groups have differences in their cell wall polysaccharide. Latex particles are coated with antibodies to these specific carbohydrates. Thus, for example, Group B will give agglutination when mixed with the latex coated with B specific antibodies, but will be negative with A, C, D, F and G latex particles. It is important to group these bacteria because they will have differing clinical significance and treatment requirements. For example, a Group A would be very significant in a wound swab, whereas a Group D (enterococcus) is more likely to be part of the resident flora.

5. Enterobacteriaceae are oxidase negative, whereas \textit{Pseudomonas} species are oxidase positive. \textit{Acinetobacter} species are oxidase negative, while \textit{Moraxella} species are oxidase positive. The oxidase test is very simple to perform yet can provide vital information when trying to identify an unknown bacterium.

6. Gram-negative diplococci could be the cause of serious infection if they are either \textit{Neisseria meningitidis} or \textit{N. gonorrhoeae}, but equally they may be the less significant \textit{N. lactamica}. \textit{Moraxella} species also appear as Gram-negative diplococci in a Gram stain. The oxidase test should be carried out first, and if positive, a choice of tests are available, such as the Gonocheck test and the API NH.

7. The urease test will distinguish between bacteria such as \textit{Proteus mirabilis} (urease positive) and \textit{Salmonella} species (urease negative). This is important in the investigation of faecal samples because \textit{Proteus mirabilis} may mimic \textit{Salmonella} species on XLD plates, with the colonies showing evidence of hydrogen sulphide production. A positive urease test will ensure that further unnecessary tests are not performed because the presence of \textit{Salmonella} species is clinically significant as a cause of bacterial gastroenteritis, whereas the presence of \textit{Proteus} species is not.

8. The main components of culture media are amino-nitrogen nutrients in the form of peptones, agar to produce the solid culture medium, glucose, other carbohydrates, buffer salts to balance the waste products and changes in pH, mineral salts, phosphates, calcium, magnesium, iron and trace elements. Growth factors such as blood, serum, vitamins and NADH are often added so that a range of clinically important bacteria will be able to grow on the medium.
9. The MIC is the minimum inhibitory concentration of antibiotic required to inhibit growth under standard conditions. The immune system is then able to eradicate the bacteria. At this concentration the antibiotics are said to be bacteriostatic. *In vitro*, if the bacteria are subcultured on to antibiotic free medium they will recover and grow. The MBC, the minimum bactericidal concentration, is the concentration at which the bacteria are killed. Some antibiotics are always bactericidal, whereas some are bacteriostatic at one concentration but bactericidal at a higher concentration.

10. The principal methods for detecting MIC *in vitro* from clinical isolates are the broth dilution test, the E test and the agar dilution method. The disc diffusion test measures the MIC indirectly by the use of regression lines and by relating the size of the zone to MICs. The results are expressed as sensitive, resistant or intermediate.

**CHAPTER 7**

1. The minimum requirements for specimen reception will be available in every laboratory in the relevant SOP, and will follow guidelines set by the Department of Health. The use of the patient’s NHS number is mandatory on the request form, in addition to full name, date of birth, address, GP or consultant and the report destination. The patient sample must match the request form and give the full name, date of birth and NHS number.

2. Timing of the specimen (e.g. before antibiotics are given); the appropriate type of specimen (e.g. sputum rather than saliva); minimum contamination by normal flora at other sites when specimens are taken from normally sterile areas of the body; sufficient quantity to avoid over-dilution in blood culture broth or to give a reasonable opportunity to isolate pathogens; use of the correct container for the test requested.

3. NEQAS is an external quality assurance scheme where identical samples are sent to all participating laboratories. The specimens should be treated in the same way as any other samples and the result is reported to NEQAS. The laboratory is then given a score. The method ensures that there is consistency across all participating member laboratories and forms part of CPA accreditation. NEQAS also provides an educational role, allowing participants to gain experience of infectious agents they may rarely, or never, encounter.

4. There are health and safety reasons for ensuring that clinical specimens are stored and disposed of in a safe manner, to protect workers and the public from contamination. There are also ethical reasons for having strict guidelines, as directed in the Human Tissue Act (2004). For some specimens, there may be medical and legal reasons to retain the specimen, for further testing or for comparison over a period of time. Examples might include serum samples for HIV, antibody levels, cases of needle-stick injury, etc.

5. The main requirements are encompassed in the Health and Safety at Work Act (1974). Employers must provide a safe place to work with safe systems, safety in the use of all substances governed by the COSHH regulations, health and safety information, instruction and training and a healthy working environment. All organizations must
display a written Health and Safety policy and supply personal protective equipment relevant to the tasks performed.

6. COSHH stands for control of substances hazardous to health. All procedures, reagents and test kits have to include a risk assessment of each chemical and reagent used.

7. Howie coats are essential to safe working and protection of the biomedical scientist, and should be worn closed to the neck and have safety cuffs on the sleeves. Access to hands-free washing facilities should be readily accessible. Disposable gloves should be worn when dealing with clinical samples and goggles available when working with liquid cultures. Facilities for the safe disposal of culture loops, etc. must be sited at the work station. Further PPE must be used when working at containment level 3 – separate gowns and gloves are provided within the room or suite and are not worn in the general laboratory area.

8. SOPs ensure consistency and quality standards for a test or procedure, ensuring that whoever performs the technique will follow the same method, interpretation of results and reporting. They are an essential part of total quality assurance.

9. New methods are introduced often because evidence from research demonstrates that the technique gives improved sensitivity, specificity and diagnostic value. There may also be a time saving element in the testing procedure. For example, research studies demonstrated that Mycobacteria grew preferentially in liquid rather than solid culture media and consequently automated systems were introduced into the laboratory. Nucleic acid amplification techniques for Chlamydia species were shown to be much more specific for detecting infection, particularly with the introduction of the new screening programme.

10. A microbiology laboratory can only work with the specimen it is given and so it is important that the specimen reflects the situation at the site of infection as closely as possible. The specimen must contain sufficient quantities of the pathogen in order to detect them in vitro; for example, a specimen of saliva will not be representative of the site of a respiratory infection, and instead of demonstrating the causative organism, will reflect only oral flora.

**Answers to case study in Box 7.2**

The correct course of action in this case would be that the faecal specimen would be discarded, and a repeat sample requested. A leaking specimen is a health and safety hazard. A faecal specimen is also easily repeated. In the case of the blood culture samples, the ward would be informed and a repeat requested. If this was refused, the requesting clinician would be asked to come to the laboratory and sign a disclaimer regarding the specimens. They would also be asked to relabel the specimen and sign that they had done so. Both samples are relatively easy to repeat and the patient is an inpatient of the hospital. The situation would be different if the patient could not easily be recalled to the hospital or if the sample itself were difficult or impossible to repeat (e.g. a CSF, a sample prior to antibiotic treatment or a wound swab taken in the operating theatre).
CHAPTER 8

1. Midstream urines, catheter urines from the collection bag, bag urine from babies, suprapubic aspirates and conduit urines are all sent for analysis and should be clearly labelled and collected into suitable sterile containers.

2. The presence of the plastic tube inside the urethra allows the normal defence mechanisms of the mucous membrane to be bypassed and bacteria are able to ascend on the outside of the catheter. The catheter itself also provides an ideal environment for bacterial growth and colonization of a mixed community of bacteria. Bacteria attach to the surface of the catheter and form biofilms from which cells are then shed.

3. The urinary tract is protected by substances secreted onto the mucosal epithelial layer (secretory IgA, lactoferrin, defensins), and in males also by the prostatic fluid. Ascending bacteria are also engulfed by phagocytic cells. In addition to this, the flushing of urine dislodges the uppermost layer of epithelial cells and the bacteria are flushed away. The presence of the resident flora is also a deterrent.

4. (a) A normal urine sample would contain very few white blood cells and some epithelial cells. (b) In contrast, an infected urine would contain large numbers of white blood cells (recruited to the area to phagocytose bacteria), possibly red blood cells (as a result of damage from the infection and the immune reaction) and possibly casts, where protein is forced through the kidneys if they are involved in the infectious process. Bacteria or yeast cells and pseudohyphae may also be seen.

5. *Escherichia coli* is a common isolate in urinary tract infections. Other coliforms including *Klebsiella* species, *Enterobacter* species and *Proteus* species are also isolated, along with both coagulase-positive and -negative staphylococci and *Pseudomonas* species, enterococci and Group B streptococci.

6. The culture media selected must be able to provide an easy identification of the principal pathogens, suppress the growth of skin flora and prevent the swarming of *Proteus* species, which even if present only in small numbers can swarm across culture media and contaminate all other bacterial growth. CLED is a good example and chromogenic agar is increasingly used in urinary culture.

7. Enzymes in growing bacteria break down substrates present in chromogenic agar. The breakdown of specific substrates releases chromophores which are incorporated into the colony formation, giving a characteristically coloured colony that can easily be identified.

8. The most likely lactose fermenting bacteria on the CLED medium would be *Escherichia coli* or another lactose fermenting coliform such as *Klebsiella* species. The oxidase test would distinguish between *Proteus* species (oxidase negative) and *Pseudomonas* species (oxidase positive).

9. Sterile pyuria is the term used to describe a urine sample where large numbers of white cells have been seen in the microscopy and no growth is observed on the culture plate. This may be because the patient has taken antibiotics prior to the submission of the specimen, in which case the bacteria may be dead. Alternatively it may be
that the bacteria causing the infection are not able to grow on the culture media routinely used for urine culture. The infection could be caused by mycobacteria from a reactivated TB infection in the bladder, in which case a request would be generated for three early morning urine samples to specifically test for TB. Alternatively, it could be caused by Chlamydia trachomatis infection, which again would require further appropriate testing with an early morning urine.

10. One of the principal factors in the choice of suitable antibiotics is the ability of the drugs to reach therapeutic levels in the bladder and urine. The choice will also be influenced by the antibiotic prescribing policy of the hospital and by the ease of administration – oral antibiotics are obviously first choice for outpatients. Isolates of Pseudomonas species are resistant to many of the first-line choices of antibiotics and will need to be tested against a wider range in order to find a suitable therapeutic agent.

Answers to case study in Box 8.1

1. Drinking fruit juice is an attempt to dilute the bacteria in the bladder and literally wash them out. Many uncomplicated UTIs will be self-limiting, and flushing out the bacteria enhances the normal immune clearance system. There is also some research to suggest that cranberry juice can help to prevent adhesion of Escherichia coli to the bladder mucosa.

2. A normal healthy urine sample would not normally contain protein or significant levels of white or red blood cells. The fact that these screening biochemical tests were positive was a further indication of infection.

3. The white blood cell count is significantly raised and suggestive of infection. The neutrophils are recruited to the infected area to engulf the bacteria. The combined activity of the immune response and the infectious process cause damage to the host tissue, resulting in the presence of red blood cells.

4. The bacteria are Staphylococcus saprophyticus, a common cause of UTI in young women.

5. The infection most probably started as an autoinfection of the lower urinary tract, affecting the urethra and bladder. Despite the large amount of fluid flushed through the bladder, the bacteria multiplied. As they did so, they ascended the ureters and caused infection in the lower parts of the kidneys, giving rise to the pain in that area and the systemic signs of infection, the raised temperature and nausea.

Chapter 9

1. Infectious agents are passed by the faecal–oral route, either by faecal contamination of food and water via poor sanitation systems or by poor personal hygiene. Contaminated food and water is then consumed by an uninfected person. Similarly, poor personal hygiene, for example lack of handwashing, allows infection to be passed from person to person. The infectious dose required to cause infection is another important factor. If the required dose is high, as with, for example, Campylobacter
species, the infection is passed less readily than an agent with very low infectious
dose such as norovirus or *Escherichia coli* O157, which can easily be passed from
person to person. Norovirus is also extremely hardy in the environment and can
therefore contaminate surfaces and utensils used by other potential victims.

2. All microorganisms with the ability to cause infection in the GI tract must be able
to survive the low pH of the stomach and be bile resistant. They must then attach to
appropriate receptors in the small or large intestine and interact with the epithelial
cells. Some have the ability to produce toxins. Protozoan parasites are protected as
cysts and develop into the reproductive forms in the GI tract, being excreted as cysts
once more. Eukaryotic ova are also protected by a tough outer coat in the external
environment. They excyst in the intestine and the larva grow into mature worms.
The transmission continues when larval cysts are excreted via the faeces into the
environment.

3. The faecal sample is cultured onto selective culture media formulated to distinguish
pathogens of interest. This could be XLD, DCA or a chromogenic agar. An enrichment
broth, designed to suppress the growth of other bacteria and enhance the growth of
the small numbers of *Salmonella* species that may be present, may well be inoculated
on the first day and subcultured on to a suitable agar the following day. Suspect
colonies are then further tested, using either an in-house biochemical identification
system or a commercial identification kit. Further typing of the bacteria is performed
using polyvalent sera for the O and H antigens, followed by specific antisera. If
*Salmonella typhi* is suspected, the capsular antigen must also be serologically tested.
Some laboratories do not carry out the O and H typing and will send the isolate to
the reference laboratory for confirmatory tests. Antibiotic susceptibility testing is
also performed.

4. The bacteria are unable to gain entry through the top of the intestinal epithelial cells
and so they pass through the M cells, escaping from and destroying the macrophages
at the base of the cells. They then induce phagocytosis from the basal surface of the
enterocytes and are taken inside the cell in a vacuole. After escaping from the vacuole
they multiply inside the cytoplasm and then move from cell to cell by the production
of actin tails that propel the bacteria. The host cells die as a result of this and are
sloughed off. Neutrophils and red blood cells accumulate in the area as a result of the
infection and damage. An ulcerated area may result, giving the classic symptoms of
bacterial dysentery. *Shigella dysenteriae* type 1 is the cause of considerable mortality
in children in developing countries. The bacteria are resistant to antibiotics and
produce a toxin, Shiga toxin, which makes the symptoms more severe.

5. The first patients were infected from eating the contaminated meat. The infectious dose
of *Escherichia coli* O157 is extremely low and so the bacteria have a high infectivity
rate and are easily passed on via person to person contact to people who had not
eaten the meat. In the very young and the elderly, the infection can be complicated by
the activity of the Shiga toxin released by the bacteria, which can lead to haemolytic
uraemic syndrome. This in turn can lead to chronic renal failure and death.

6. *Giardia lamblia* are protozoan pathogens, existing in two forms, the environmentally
protected and inert cyst and the trophozoite form, in which replication takes place
in the intestines of humans and animals. Cysts are shed by animals into water (often water used as a drinking water source). Humans are infected either by water/food or directly from person to person contact with an infected individual. The cyst is ingested and passes through the stomach where contact with the gastric acid enables the cysts to change into the trophozoite (flagellate) form. Motile flagellates attach to the mucosal cells in the duodenum and jejunum by their sucking disc. The attachment hinders the normal absorption processes and the patient suffers from dehydration, fatty stools, diarrhoea and excess gas. The flagellates replicate and pass out of the body as cysts.

7. Each gram of faeces contains a very high number of bacteria, and even though the majority of the bowel flora do not grow aerobically, there are still considerable amounts of facultative anaerobes that do produce colonies on culture plates, and most of these are Gram-negative bacilli. It is therefore very important to be able to distinguish the normal Gram-negative flora from the relatively small numbers of pathogens, such as salmonellae and shigellae, that may be present. Selective media contains bile salts to inhibit the growth of normal flora and sufficient nutrients to encourage the growth of the pathogens. Biochemical differences are then exploited by the addition of carbohydrates, or chromophores that will provide visual colonial differences and allow the suspect pathogens to be picked out and further tested. One example of a selective medium is DCA that contains bile salts and lactose. Fermentation of the lactose lowers the pH which is indicated by the addition of phenol red. The faecal pathogens *Salmonella* species and *Shigella* species do not ferment lactose and appear as colourless colonies among the commensal lactose fermenting pink colonies.

8. Norovirus has a very low infectious dose and therefore a high attack rate. It is also able to survive for periods of time on hard surfaces. The problem with a cruise ship is that first of all the source of the infection has to be identified and disinfected or isolated; this could be the ship’s water supply or it could be an infected person in the kitchen, or a passenger. However large the ship is, everyone is obliged to remain living on it and the infection can spread from person to person very easily; with large numbers of people with projectile vomiting, this poses an infection control problem. On more than one occasion, a cruise ship has had to return to port as the result of a norovirus infection.

9. If the patient has travelled to India and Nepal they could have been exposed to a variety of parasitic infections and there is also a risk of cholera and typhoid fever in addition to the more common infections. Culture media for the identification of *Vibrio* species would be inoculated to identify possible *Vibrio cholerae* or *V. parahaemolyticus*. The former would grow as yellow colonies on TCBS agar and the latter as green colonies. A concentration of the faecal specimen would be examined for the presence of ova and cysts. A stained smear would identify the presence not only of *Cryptosporidium parvum* but also some of the more unusual cysts of *Cyclospora* and *Isospora* species. If suspected salmonellae are isolated, they must be checked to exclude the presence of *Salmonella typhi*.

10. There are two important reasons for the timely identification of *Clostridium difficile* infection. First, the diagnosis is important for management and treatment of the
patient, who may be developing serious antibiotic-associated disease. Their antibiotic regime needs to be changed to treat the infection and prevent further damage. Secondly, the diagnosis is important in terms of infection control and may result in patients being isolated and barrier nursed. Infection control measures to limit the spread of the disease must be implemented.

**Answers to case study in Box 9.2**

1. *Campylobacter* species, because it is oxidase-positive and a curved bacterium requiring incubation on specialized media in micro-aerophilic conditions.

2. Food containing the bacteria, such as raw or undercooked poultry or minced beef such as beefburgers, unpasteurized milk and cheese made with unpasteurized milk, or contamination of another food by contact with raw poultry, etc.

3. Obviously it is important to treat the patient appropriately. There is also evidence of resistance to ciprofloxacin, a fluoroquinolone, among *Campylobacter* species. This may be due to overuse of the antibiotic; exposure leads to a point mutation in the bacterial DNA gyrase, leading to resistance. The use of similar antibiotics in animal husbandry may also cause cross-resistance, as may visits to countries with high fluoroquinolone resistance levels.

4. Until relatively recently, *Campylobacter* infections were not diagnosed because the bacteria could not be cultured. The use of specific selective agar is necessary as well as incubation in suitable micro-aerophilic conditions. Accordingly, the bacteria will not grow on the routine selective agar used to isolate bacterial faecal pathogens in aerobic conditions.

**CHAPTER 10**

1. The skin is a potentially challenging area for exogenous bacteria because of its well-developed defence system including the resident flora, the presence of immune cells and the hostile environment it presents for would-be pathogens. The normal flora occupy all the attachment niches and so exogenous bacteria have to find a way to overcome them. The area is well protected by antibiotic peptides, Langerhans cells and lymphocytes. The physical environment is challenging because the top layer of cells is continually being shed and the area contains salt, sweat and fatty acids. However, bacteria overcome these natural defences by sheer force of numbers, or more frequently by entering the lower levels of the skin via cuts and bites, ulcers and foreign bodies including instrumentation such as intravenous lines.

2. Necrotizing fasciitis describes an infection of the fascial layers below the skin (the fat layers, soft tissue and superficial fascia). Fortunately the disease is rare, but can have devastating consequences because the bacteria involved switch on powerful toxins and destroy the tissue, creating an area of necrosis. Spread of the infection is swift, and rapid effective antibiotic treatment is required, in addition to surgical debridement of the tissue. Type I necrotizing fasciitis is caused by a mixed infection of an anaerobe, such as *Bacteroides* species and a facultative aerobe, such as a member
of the Enterobacteriaceae or streptococci, but not *Streptococcus pyogenes* as this is implicated in type II. Type II is typically caused by *Streptococcus pyogenes* (Group A streptococcus) and its powerful toxins SPE-A, B and C. *Staphylococcus aureus* is less commonly the causative organism, but can cause serious disease involving powerful toxins, including Panton–Valentine leukocidin.

3. For a deep post-operative wound a range of culture media need to be inoculated to cover aerobic and anaerobic bacteria and to differentiate between lactose fermenting and non-lactose fermenting coliforms. Blood agar would be included, as would an anaerobic agar and a CLED or MacConkey agar. The bacteria may be present in low numbers and so an enrichment broth should be included to encourage their growth.

4. It is important to know the age and some clinical details accompanying a genital specimen from a female because the normal flora and likely pathogens are influenced by age and behaviour. Before and after the onset of menstruation there are fewer lactobacilli in the vaginal area. The presence of these bacteria maintains the pH at a slightly acidic level and inhibits infection from exogenous bacteria. Children do not have this protection and therefore are susceptible to a wider range of genital infections. After the menopause infection with coliforms and bacteria vaginosis are more common. If the patient is in the latter stages of pregnancy the presence of group B streptococci may be significant. If the clinical details suggest that a sexually transmitted disease may be the cause of the symptoms, appropriate agar for gonorrhoea can be inoculated. Similarly in an adult female a smear is examined for the presence of *Trichomonas vaginalis*.

5. *Neisseria gonorrhoea* bacteria are cultured and identified on specialized media and should be inoculated on to the media as soon as possible after the specimen is taken. The bacteria are very fragile outside the human body and are fastidious in their growth requirements. Special media containing antibiotics to discourage the growth of other bacteria, and special growth supplements to enhance the growth of *Neisseria gonorrhoea* are used. After culture, suspect colonies are identified by Gram stain, appearing as Gram-negative diplococci, and by a positive oxidase test before confirmation using either an API NH test strip or by the Gonocheck system. Susceptibility tests are also performed on the isolate to ensure that appropriate treatment is given. The infection can also be diagnosed by using a molecular amplification method.

6. The significance of culturing Group B streptococci from a female genital specimen is influenced by the clinical status of the patient. It is often present alongside *Candida albicans* infection and does not require treatment. Small amounts of the bacteria growing as part of the normal vaginal flora are also not clinically significant. Its significance increases when the patient is in the later stages of pregnancy or in labour, because the baby may be infected as it passes through the birth canal. However, the incidence of neonatal infection is low, because serious infection is caused by certain serotypes of the bacteria and also when there is a lack of maternal antibodies.

7. The pathogenic bacteria most commonly isolated from eye infections are normal members of the skin flora, staphylococci and *viridans* streptococci. Infections are also caused by *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Pseudomonas*
aeruginosa. Eye infections in newborn infants can be caused by Neisseria gonorrhoeae, Chlamydia trachomatis or Treponema pallidum.

8. It is important because the two conditions differ in severity and in their treatment. Otitis externa is an infection of the outer ear (the outer auditory canal), and can be acute or chronic. The pathogens involved are usually similar to those causing skin and soft tissue infections – *Staphylococcus aureus* and Group A streptococcus, and *Candida albicans*. *Pseudomonas aeruginosa* is also a frequent pathogen, often acquired from swimming. The infection can be treated with topical antibiotic cream. Otitis media is a much more serious condition causing acute illness. It is caused by the presence of fluid in the middle ear via the Eustachian tube, which brings oro-pharyngeal flora into the area. The principal pathogens are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, group A streptococci and *Moraxella catarrhalis*. Infections are more common in children and must be treated as soon as possible with oral antibiotics to prevent hearing loss.

9. The most frequent pathogen to be isolated in cases of acute tonsillitis is Group A streptococcus (*Streptococcus pyogenes*). The bacteria grow easily on blood agar or staph/strep agar and are distinguished by an area of beta-haemolysis around the colonies. However, this would be confirmed by Lancefield grouping of the isolate. A bacitracin disc is often added to the plate prior to incubation; Group A streptococci are sensitive to bacitracin and there will be a clear zone of inhibition around the disc.

10. It is necessary to inoculate a greater number of culture media for an ear swab than a throat swab because ear infections are caused by a wider range of pathogens. These include *Haemophilus influenzae*, and so a chocolate plate is inoculated. A CLED or MacConkey plate is also inoculated to distinguish *Pseudomonas aeruginosa*, a pathogenic non-lactose fermenter from normal lactose fermenting coliforms present in skin flora. A Sabouraud agar to identify any fungal pathogens is also inoculated. Anaerobic bacteria may also be the cause of ear infections and therefore an anaerobic culture medium is also included.

**Answers to case study in Box 10.3**

1. A blood agar or staph/strep agar for good growth and demonstration of haemolysis. A CLED / MacConkey agar to differentiate lactose fermenters from non-lactose fermenting coliforms. A blood or specific anaerobic plate for the presence of anaerobic bacteria.

2. *Staphylococcus aureus*, β-haemolytic streptococcus. The anaerobic bacteria would also be reported.

3. The identity of the *Staphylococcus aureus* would be confirmed by a DNAse plate or another suitable chromogenic plate. Antibiotic susceptibilities would be performed, for treatment guidance and to demonstrate that the isolate was not MRSA. The β-haemolytic streptococcus would be Lancefield grouped to demonstrate that it was in fact a Group A, and antibiotic susceptibilities would be performed.

4. The presence of these bacteria would prevent the normal healing process because
they both produce toxins and enzymes that break down tissue and further contribute to the ulcer formation.

5. Immobility, poor tissue perfusion, spontaneous breakdown of the tissue, trauma to poorly perfused tissue and underlying conditions such as diabetes can all be contributory factors.

CHAPTER 11

1. The respiratory tract defences start as soon as air is inhaled through the nose, with the presence of nasal hair and mucus-covered membranes. The tonsils in the oro-pharynx aid in the production of antibodies to foreign antigens, particularly in children, and form part of the lymphoid defence system. Ciliated epithelial cells and mucous membranes protect the lower respiratory tract, by trapping microorganisms in the mucus and propelling them upwards in the cilia, where they are either swallowed or expelled from the upper respiratory tract. The mucus contains IgM antibodies, lactoferrin and defensins and the surface of the epithelial cells is covered in normal flora which provides colonization resistance. However, members of the normal flora can also act as pathogens if they gain access to the bronchial tubes and the lung, which are normally sterile areas. The lungs are protected by the pleura, a double-layered membrane enclosing them.

2. The old and the very young are more at risk because their immune systems are less efficient or less developed. Certain occupations expose people to different respiratory pathogens, for example farmers, veterinary surgeons, rodent controllers, drum makers and others working with imported animal skins, owners of birds, and anyone exposed to aerosols containing *Legionella* species; and also occupations where workers are exposed to dust. Many individuals have chronic lung disease that impairs their lung function and airways. They are more prone to acute flare-ups (exacerbations) of acute bronchitic infection. Smokers and alcoholics are also more prone to respiratory infection. Cystic fibrosis sufferers suffer from respiratory infection at intervals throughout their life as a result of the specific conditions of their lungs. Hospital patients are at risk of lower respiratory infection either because of their underlying illness or as a result of loss of consciousness, instrumentation (for example, ventilators), or treatment regimes that render them immuno-compromised. Immuno-compromised patients are a further risk group and prey to opportunistic respiratory tract infection.


4. The success of microbiological diagnosis depends on the quality of the specimen received, which should represent as closely as possible the conditions at the site the infection. A specimen of saliva will only represent oral flora, whereas a genuine specimen of sputum will be expelled from much lower down the respiratory tract and will more accurately represent the infection at that point. In a case of TB, the infection may be in an area of the lung that does not communicate as well with the bronchi and upper respiratory tract. The bacteria may also be excreted in low
numbers and so three specimens are requested to give the best opportunity of isolating the relevant bacteria.

5. Some cases of atypical pneumonia are caused by bacterial pathogens that cannot be cultured, e.g. *Mycoplasma pneumoniae*, *Chlamydophila psittaci*, *Chlamydia pneumoniae* and *Coxiella burnetii* are diagnosed by either molecular techniques or serology. This is true of viral pathogens and unusual fungal pathogens which are also diagnosed by molecular methods.

**Answers to case study in Box 11.4**

1. The cause of his pneumonia was most probably *Streptococcus pneumoniae*, despite his being immunized. At that age the immunity given by the vaccination was probably insufficient to prevent the infection of already diseased lungs.

2. He could have become infected with members of the normal flora, for example *Moraxella catarrhalis* or *Haemophilus influenzae*, or even oral flora accidentally aspirated into the lung area. Because of the chronic emphysema and impaired lung function, *Pseudomonas aeruginosa* could have been present. No sputum sample was taken and so the causative organism was not identified. Because the X-ray showed a distinct area of infection the cause was most likely to be bacterial, rather than the diffuse picture of viral pneumonia.

3. The predisposing factors were undoubtedly age, declining immunity and the pre-existence of chronic pulmonary disease. Emphysema affects the elasticity of the lungs and makes it more difficult to expel air efficiently. During the winter months there would be a greater number of respiratory viruses circulating, affecting staff within the care home. Infection with a common virus could have increased his susceptibility to bacterial pathogens.

4. The sputum would be treated with a mucolysing agent to release the bacteria. The specimen would then be inoculated on to blood, chocolate and either CLED or MacConkey agar. This combination of blood and chocolate culture media would detect the most likely bacterial respiratory pathogens and the CLED or MacConkey agar would demonstrate whether any coliform bacteria present were lactose or non-lactose fermenters. This is significant because a possible pathogen would be *Pseudomonas aeruginosa*, which is non-lactose fermenting and oxidase positive. After overnight incubation of the culture media at 37°C, the plates would be examined for the growth of significant pathogens. *Streptococcus pneumoniae* would appear as mucoid or drier colonies resembling the pieces in a draughts set, and are sensitive to an optochin disc placed on the plate to confirm the presence of these bacteria. The disc could either be added during primary culture or used as a confirmatory test when the colonies were subcultured. The Gram stain would show lanceolate Gram-positive cocci. *Haemophilus influenzae* would show enhanced growth on the chocolate agar and reduced growth on the blood. *H. influenzae* are oxidase-positive small Gram-negative rods. The X and V test to confirm the presence of *H. influenzae* would show growth only around the XV disc. *Moraxella catarrhalis* are Gram-negative cocci, and are oxidase positive. All isolates should be tested against a range of antibiotics to check that the antibiotic administered to the patient was effective.
Answers to case study in Box 11.5

1. The details accompanying the first sputum test did not specify that TB was a possibility and so the sample would be cultured on routine agar which would identify common bacterial pathogens, but not TB.

2. The mycobacteria may be present in small numbers only, and depending on which part of the lungs were infected, may not be expelled in large numbers either. By requesting more than one sample, the chances of isolating the bacteria are increased.

3. This is likely to be a reactivated case of latent TB. When F. caught the initial infection his immune system was strong enough to isolate the bacteria, leaving him as one of the millions of people with latent TB that can be reactivated at a later date.

4. F. was a coal miner and had been exposed to coal dust over a number of years which will have made him more susceptible, coupled with the fact that he is now older and his immune system is weaker. Mycobacteria are slow growing and therefore have the potential to develop resistance if treated with a single drug or for an insufficient period of time. Current therapy involves a combination of drugs taken over a period of several months: isoniazid, rifampicin, ethambutol and pyrazinamide. After the initial phase of 2 months, treatment is continued with isoniazid and rifampicin for a further 4 months.

5. The speed with which the laboratory report was issued suggests that tubercle bacilli were visualized in the smear prepared from the sputum prior to inoculation of the culture media, whichever the culture method used. Confirmation of the culture of the bacteria would be sent once the bacteria had either formed visible colonies on solid agar or flagged positive on the automated machine and confirmed by staining a preparation from the liquid agar. Antibiotic sensitivities would be carried out at the reference laboratory. The patient would be treated with antibiotics on diagnosis because the results would take several weeks. Again, because mycobacteria are slow growing, they also take time to give antibiotic susceptibility results.

CHAPTER 12

1. Large quantities of lipopolysaccharide are released from Gram-negative cells when they die, the lipid A being particularly toxic and immunogenic for humans. In the normal innate immune response, acute phase proteins are released from the liver and neutrophils are recruited to the site of infection. The cascade systems of the clotting, complement and arachidonic acid are activated and the infection is controlled, the patient suffering symptoms of raised temperature, myalgia and inflammation. If the bacteria are present in overwhelming numbers, large quantities of lipid A are released, leading to an exaggerated response by the immune system. Large quantities of cytokines are released by macrophages after they have been stimulated by the binding of LPS binding protein bound with lipid A to their CD14 receptor. These high levels of circulating cytokines can then lead to leaky small blood vessels and hypovolaemic shock. This may then lead to multi-organ failure and death.
2. Bacteria from the oral cavity may gain access to the circulation and lodge on the faulty or prosthetic heart valve. They attach to the foreign surface and proceed to form a biofilm. Bacterial cells are then shed into the bloodstream, causing a rise in temperature and bacteraemia.

3. Growing bacteria produce carbon dioxide that is detected by sensors at the base of the blood culture bottles. The level of reflected light is measured every ten minutes and a curve of the reflected signal is produced after it has been analysed by the software. A positive bottle will produce a characteristic upward curve that will alert the system to flag the bottle as positive once it has reached a preset threshold.

4. The presence of bacteria in the blood culture bottle may not necessarily reflect the clinical state of the patient. For this reason it is important that the result is interpreted by someone who has first-hand knowledge of the patient’s condition. The presence of a skin organism may represent contamination of the blood culture when it was taken, or may be a genuine infection if the patient has an infected cannula.

5. Gram-positive cocci will need further identification to species level if they are significant isolates. Growth on a suitable culture medium is the first important step, allowing colonial morphology to be examined, e.g. size and nature of colony, whether alpha or beta haemolytic, draughtsman-shaped colonies of Streptococcus pneumoniae or the typical colonies of staphylococci. A catalase test will distinguish between streptococci and staphylococci if necessary. If it is a beta-haemolytic streptococcus, Lancefield grouping will follow; if it is an alpha-haemolytic streptococcus then tests for Streptococcus pneumoniae and further identification by API strep or Vitek technology. For staphylococci, the coagulase and DNA (or other identification plates for Staphylococcus aureus) are necessary, followed by API staph or Vitek confirmation. In addition, all isolates require testing with suitable antibiotics to guide therapy and also to check for MRSA.

6. The CNS is distinctive because it is well protected and isolated by the skull and blood–brain barrier, but at the same time highly vulnerable to serious damage. Because of the rigidity of the skull, minor swelling and inflammation are magnified. The inflammation causes significant damage, with the increased pressure and possible involvement of the brain tissue leading to further complications and systemic effects. The passage of microorganisms and toxic substances into the brain is normally inhibited by the blood–brain barrier. This is also true for antibodies and cellular defence components and only certain antibiotics are able to pass through. Despite being well protected, the central nervous system is a relatively hidden area with fewer defence mechanisms than other systems. Complement levels are low because of partial inactivation by the CSF and also because of poor penetration of the blood, and phagocytosis and lysis of bacteria do not occur to the same extent in the brain, meninges and CSF. The microglial cells provide some immunosurveillance and have many surface markers in common with circulating blood monocytes. The Virchow Robin spaces, which are the perivascular sheaths surrounding blood vessels as they enter the brain, have a lymph-like system containing macrophages and lymphocytes, should infection arise.

7. Bacteria, viruses and fungi enter the CNS via the blood circulation and some viruses, such as rabies and HSV, enter by the neural route. The choroid plexus, where the
CSF is formed, is highly vascular and is thought to be the area where microorganisms penetrate the blood–brain barrier. Bacteria gain access into the brain tissue itself in the formation of abscesses, via the blood circulation.

8. In meningitis caused by *Neisseria meningitidis* the bacteria gain access to the meninges from the back of the pharynx and cross the blood–brain barrier. They multiply in the meninges and cause the classic symptoms of meningitis. In meningococcaemia the bacteria are present in large numbers in the blood, leading to septicaemia. The increased levels of circulating cytokines affect the clotting system, causing disseminated intravascular coagulation. The presence of the small thrombi in the blood vessels causes infarction, which may lead to subsequent amputation of fingers and toes. Septic shock and multi-organ failure are further complications.

9. The likely pathogens in this case are viruses (enteroviruses in children and more commonly HSV in adults). The presence and identity of the viruses is confirmed by either cell culture techniques, or more commonly molecular methods, such as specific PCR.

10. The introduction of the vaccines for protection against *Haemophilus influenzae* (Hib), meningitis C and *Streptococcus pneumoniae* have significantly changed the epidemiology of meningitis in the UK.

**Answers to case study in Box 12.2**

1. The culture media inoculated must be able to grow the principal pathogens likely to be present in the clinical sample; in this case a blood agar, chocolate agar and an anaerobic culture plate would be used. If the Gram stain shows the presence of a yeast, Sabouraud or similar agar would be added. If there is suspicion of an unusual pathogen such as *Mycobacterium tuberculosis*, appropriate liquid culture media would be inoculated.

2. Group F streptococci rarely cause meningitis, but along with staphylococci are more likely to cause brain abscesses.

3. The white cell count would be predominantly lymphocytic in nature, rather than neutrophilic. Bacterial infections attract neutrophils to the site of infection for phagocytosis. Viral infections attract a lymphocytic response to attack virally infected cells.

4. The patient may have had a chronic local infection of his middle ear or sinuses. He may have a history of congenital heart disease; he may have had acute meningitis in the past or some trauma to the brain. Abscesses are often associated with past episodes where the area of the brain has suffered infarction or decreased blood supply. Cell death then leads to a softening of the tissue, a condition called encephalomalacia. Bacteria carried in the blood then lodge in the affected tissue and an abscess can develop.

5. In this case the diagnosis would most likely be that of meningococcaemia and meningitis, caused by *Neisseria meningitidis*. The response to the presence of the bacteria and released endotoxin in the blood leads to an immune response involving
dysfunctional clotting mechanisms and loss of thromboresistance. This is manifested as a characteristic non-blanching rash.

CHAPTER 13

1. Many fungal pathogens can behave as moulds or yeasts during an infection within a human host. This is both temperature dependent and induced by adaptation from being free-living to life as a parasite. Some fungi will form hyphae at environmental temperatures but once within the human body will change to yeast cells. Examples of this dimorphic behaviour can be seen during an infection with *Coccidioides immitis* and *Histoplasma capsulatum*. Both are inhaled as arthroconidia but subsequently become yeast-like forms within the tissue. Both cause serious systemic disease in the host. The yeast *Candida albicans* shows the reverse behaviour, exhibiting yeast-like forms on the mucosal surfaces of the host but forming mycelia if it becomes more invasive and pathogenic.

2. The three genera of dermatophytes responsible for cutaneous mycoses are *Epidermophyton*, *Microsporum* and *Trichophyton*. The characteristic skin lesion is called a ringworm because as the infection moves out from a central point it provokes an inflammatory response (a delayed hypersensitivity reaction) in the form of a raised red scaly area that resembles a circular worm under the skin.

3. If the patient is immuno-compromised they will not be able to mount an appropriate immune response to a fungal infection. They are therefore much more susceptible to opportunistic infections that would be efficiently controlled by a fully functioning immune system. Sometimes fungal isolates from an immuno-competent patient could be classed as environmental contaminants or deemed insignificant, whereas they could be causing serious clinical problems to an immuno-compromised patient.

4. Samples of skin, hair and nail clippings are the most common specimens for the identification of dermatophyte infections, as they infect superficial layers of keratinized skin.

5. The two separate types of agar, one containing cycloheximide, for example dermasel agar and one without, such as Sabourauds, are used to distinguish between dermatophyte and non dermatophyte infections. Non dermatophytes cannot grow in the presence of cycloheximide whereas the dermatophytes are unaffected by its presence. This provides a good basis for identification, along with the microscopy and the colony growth description.

6. Dermatophytes take longer to grow; up to three weeks of incubation is common before colonies are visible. Non-dermatophytes are much faster growing. Growth on media containing cycloheximide is more likely to be a dermatophyte. The colour and texture of the colonies from the upper and reverse of the culture plate are observed. Microscopic identification is aided by noting the type of hyphae present (whether it is septate or non-septate), and the presence and shape of macro- and microconidia. Used in conjunction with the colours of the upper and reverse of the colonies, and a good reference source, a presumptive identification can be made.
7. *Cryptococcus neoformans* is a capsulated yeast, the cause of cryptococcosis. This is a subacute or chronic infection of the lungs and central nervous system, most commonly seen in immuno-compromised patients. The yeast cells can be observed microscopically, and the capsule can be clearly seen if the preparation is stained with Indian ink. In a case of meningitis the yeast cells are visible in the CSF specimen. They are fast growing and can be cultured on cornmeal Tween 80 agar at 25°C for 72 hours, when they are visible as slightly pink or yellow colonies. If grown on birdseed agar they produce brown colonies. The yeasts are urease positive and will grow in the presence of cycloheximide.

8. Fungi are eukaryotic cells and therefore it is more difficult to develop antifungal strategies, because there are fewer differences between human cells and fungi and therefore fewer targets for selective toxicity. However, where there are differences these become the target of treatment strategies. Examples of these include ergosterol in fungal cell membranes, RNA synthesis and interference with the supply of nutrients reaching the fungal cells.

9. a) Cutaneous mycoses can be treated topically with creams or with oral drugs. Griseofulvin is an oral treatment used for skin and nail infections. The drug has to be taken over an extended period of time as it is slow acting. Griseofulvin accumulates in the stratum corneum, forming a barrier which gradually starves the fungi of nutrients. Nystatin is used to treat candida infections such as oral thrush, while fluconazole is used for vaginal infections.

b) More powerful drugs are used to treat more serious and systemic fungal infections. Amphotericin B, given intravenously, binds to sterols with a greater affinity for ergosterol present in fungal membranes than cholesterol in human membranes, but there is still some toxicity for human cells associated with the treatment. Side-effects can be unpleasant and include damage to renal tubules. The azole drugs are more frequently used and can be administered orally. They also inhibit ergosterol synthesis but are less toxic. Examples of azole drugs are fluconazole, ketoconazole and itraconazole. 5-fluorocytosine is another drug treatment used for *Cryptococcus neoformans* infections. The active compound within this drug disrupts protein synthesis and inhibits fungal DNA synthesis.

10. Tinea pedis describes a dermatophyte (ringworm) infection of the foot, commonly known as athlete’s foot. The most likely pathogens involved are *Trichophyton rubrum* or *T. mentagrophytes*. Both are easy to culture and identify microscopically as they have characteristic growth; the former develop red colonies, the latter have characteristic ‘whorls’ (spirals) of hyphae.

**Answers to case study in Box 13.1**

1. *Candida albicans*, because of the colonial appearance, Gram stain, germ tube formation and the positive blue colonies on the chromogenic agar.

2. The patient has a breach in the intact skin covering because of the intravenous catheter, allowing normal skin flora to gain access to subcutaneous layers and the bloodstream. She also has a depressed immune system because of the chemotherapy
and underlying illness, leaving her with an inadequate number of neutrophils and increasing the risk of opportunistic infection.

3. Because the yeast cells have caused a systemic infection (*Candida fungemia*), they will require treatment with an azole drug such as fluconazole, ketoconazole or itraconazole given orally, or with intravenous amphotericin B.

4. Vaginal candidiasis (thrush), skin and nail infections, oral thrush.

5. Treatment with broad-spectrum antibiotics reduces the levels of normal bacterial flora without killing the yeast cells, allowing the latter to overgrow. Immunosuppression as a result of HIV infection allows overgrowth of yeast cells. Chronic mucocutaneous candidiasis is a chronic infection of the mucous membranes, hair, skin and nails in patients with T cell defects. Patients with diabetes are more susceptible to infections in the oropharyngeal area. Drug addicts are more susceptible to *Candida* endocarditis.

**CHAPTER 14**

1. Freezing affects the growth cycle by altering the membrane and enzymic activity of the cells. At low temperatures the cell membranes gel and become less fluid. Transport processes across them are so slow that growth cannot take place. Temperature is one of the most important environmental factors affecting the growth curve of bacteria. Most bacteria associated with human infection are mesophiles, growing preferentially between 9°C and 40°C.

2. The difference between HACCP and end point testing is essentially that an HACCP system quality controls and audits the whole process from raw ingredients through to completion of the product. Critical control points identified throughout the process are monitored and recorded, for example quality of ingredients and quantities used, temperature and cooking time. End point testing does just that, testing of the final product. If performed without an HACCP procedure, the reason for test failures is harder to pinpoint.

3. It is important to homogenize food samples before testing them because contamination of the food is rarely spread evenly throughout the sample. If only one small area was tested, the bacteria may not be on the surface spread on to the media. Because only a small sample is tested, the section chosen (for example of a sandwich) must represent all the ingredients. Homogenizing this weighed sample gives the best opportunity of releasing any pathogens present into an enriched fluid medium and thus the best opportunity of culturing and identifying them.

4. A resuscitation step is included in many testing protocols because pathogenic bacteria may be present in the specimen. However, they may be stressed and unable to grow in the presence of other ingredients and preservatives present in the food product. An example is the presence of salmonella in a dried milk sample. The low level of available water in the sample prevents the bacteria from reproducing. Resuscitation for a period of time in a suitable resuscitation liquid medium, such as Rappaport
Vassiliadis soya peptone (RVS) or Muller-Kauffmann tetrathionate novobiocin broth (MKTTn) for serotypes that would be inhibited by RVS broth. If the bacteria are present in low numbers the incubation in resuscitation broth allows the bacteria to recover and reproduce and therefore helps in their isolation and identification.

5. Mains drinking water is treated with chlorine and tested by the supplier. Before distribution, drinking water is tested for *Escherichia coli*, coliforms and enterococci. UK and European legal requirements state that drinking water must be free of *E. coli* and enterococci at the consumers’ taps and free of total coliforms and *E. coli* at the treatment works and service reservoir. If mains water is tested in response to a complaint it is tested for coliforms, *E. coli*, enterococci and a total viable count. Aircraft water is held in a tank rather than on a distribution system from the water supplier, and so could additionally be contaminated with *Pseudomonas aeruginosa* which can grow in untreated water, in biofilms and in water that is stored in tanks before use. Aircraft water is therefore tested for the same bacteria as mains water, with the addition of *Pseudomonas aeruginosa*.

6. Spa pools are tested for *Legionella* because the conditions are ideal for the growth of the bacteria if they are not sufficiently controlled by the chlorination treatment. The water is at an optimum temperature (30–40°C) for their growth, and jets in the system provide the characteristic bubbles in the pool. Aerosols are created in which the bacteria can be inhaled by bathers. Other sources of the infection include cooling towers which are an integral component of air conditioning systems, with tanks usually situated on the roofs of buildings. The water in the tanks cools the hot pipes from the heat exchange system, and in the process steam is formed. *Legionella* and other bacteria live and multiply in the warm water and form biofilms and can be emitted from the tank in the form of aerosols.

7. *Pseudomonas aeruginosa* can enter the swimming pool through pipes feeding the pool and in biofilms in these pipes. If they are present in sufficient numbers they can cause ear infections in bathers as they dive into the water and the bacteria are forced into their ears. *Pseudomonas aeruginosa* can also cause infection in cuts, abrasions and burns.

8. ACCs are most beneficial if they are performed routinely over an extended period of time because they act as an indicator of change in the quality of the water. Any change in the ACC may suggest that the water has become contaminated or that there is a treatment failure.

9. Total viable counts are useful indicators of the overall number of microorganisms present in the food, and therefore give an indication of the microbiological quality of the food. Food is considered unwholesome if the viable count is abnormally high and may indicate contamination of the raw materials or unsuitable time and temperature of the storage conditions. High levels of spoilage bacteria will alter the appearance and flavour of food and may also indicate the presence of food-borne pathogens. It is important to understand the nature of the food and the acceptable level of the TVC, as some natural products have a higher normal level of harmless bacteria than others.

10. The presence of *Escherichia coli* O157 is detected by testing 25 g of the food sample,
which is homogenized with 225 ml of pre-warmed modified tryptone soya broth. The bacteria are removed from the sample using an automated immunomagnetic bead separation (AIMS), where the beads are coated with anti-\textit{E. coli} O157 antibodies. The presence of the bacteria is achieved by subculture to cefixime tellurite sorbitol MacConkey agar, and incubation at 37°C for 24 hours. Most strains of \textit{E. coli} O157 are non-sorbitol fermenters and appear as clear colonies. However, there are emergent strains that are sorbitol fermenters. Five colonies are subcultured on to MacConkey agar and nutrient agar. The bacteria grow as lactose fermenting colonies on the MacConkey agar and confirmation is achieved by latex agglutination tests from nutrient agar plates. \textit{E. coli} O157 bacteria have been associated with several serious outbreaks where patients have died or suffered serious illness (haemolytic uraemic syndrome, HUS) from the effect of the bacterial verocytotoxin. The presence of the bacteria in food is therefore significant as the infectious dose is very low. Although associated with undercooked beef and dairy products, \textit{E. coli} O157 infections have originated from a variety of different foods. Because of the highly infectious nature of the bacteria, testing of isolates is carried out at containment level 3. The presence of the bacteria in a sample of food is always significant.