1

Overview of Clinical Diagnostic Microbiology

1.1 Introduction

The scope of diagnostic microbiology has developed along with technological advances in laboratory science. In the nineteenth century, detection of organisms relied on light microscopy and a limited range of *in vitro* culture methods. Some of these techniques are still used to detect and identify bacteria and parasites, in some cases with little or no modification (e.g., Gram's stain). The discovery of proteins and then later nucleic acids during the twentieth century, along with the advent of the electron microscope, opened up the microbial world. The ability to determine and characterise the exact cause of an infectious disease, and thus to devise control measures, treatments, and prophylaxes has reduced morbidity and premature mortality throughout the world. Indeed, some diseases – smallpox in humans and rinderpest in cattle – have been eliminated altogether.

The ever-expanding range of known microorganisms has led to the separation of diagnostic microbiology into distinct specialist areas – bacteriology, virology, mycology and parasitology. The diversity of microorganisms necessitates the training of individuals to be experts in one particular type of organism and they are often considered separately during study. It is important to remember that microorganisms do not operate in isolation and that a patient can be infected with more than one pathogen simultaneously. Advances in technology, in particular automation, make it possible to test for markers across pathology disciplines (not just within microbiology) on one single specimen in relatively short turnaround times. This means that the diagnostic microbiologist must be aware of the implications of an eclectic mixture of results and should certainly be able to solve problems and make decisions about patients with a wide range of infections.

N.B: This book assumes microbiological knowledge to at least graduate level. There are some very good general textbooks available for those who would like to revise any areas. For example, Greenwood *et al*. (2012) provides a brief overview of a comprehensive range of organisms, while clinical features are addressed clearly and simply in Murray *et al*. (2013) and Goering *et al*. (2013). Laboratory aspects are covered by Ford *et al*. (2014) and Wilkinson (2011), which were both written by practising diagnostic microbiologists.

Point to consider 1.1: What do you think is the most important scientific discovery in microbiology during the last 100 years and why?

Clinical Microbiology for Diagnostic Laboratory Scientists, First Edition. Sarah J. Pitt.

1.2 Organisation and Management of Diagnostic Microbiology Services

The configuration of pathology services seems to be constantly changing in response to management trends, as well as financial constraints. Technological advances mean that it is easier to identify microorganisms to the level of a strain within a species, in a much shorter amount of time than it was 30 years ago. The techniques available have also been developed such that many aspects of the laboratory work can be done in automated systems (e.g., Fournier *et al*., 2013). The graduate and postgraduate microbiology scientist must therefore be able to interpret ever more complicated data, while retaining a working knowledge of the principles behind the tests used to generate that information. As laboratory microbiologists develop expertise and are given more responsibility, they sometimes find themselves working in larger teams, while at other times they are concentrating on a specialised area with a small number of colleagues. Cooperation with staff across pathology and in other departments in the hospital and primary care is becoming increasingly important in the daily running of the diagnostic microbiology service. Also, responding to the requirements of external bodies such as those involved in public health surveillance, quality assessment and service monitoring can create enough work for a full-time post. Employing organisations can come and go, while the people who staff the laboratories often stay the same. This can be disconcerting, but it is important to keep sight of the facts. Firstly, each sample received in the laboratory has been collected from a patient with a clinical problem which needs addressing such as, 'Has there been an adequate immunological response to this course of a vaccine?', 'What is causing this rash?' and 'Why does this antimicrobial treatment appear to be ineffective?' Secondly, microbiology is endlessly fascinating; organisms can change very quickly – rendering diagnostic methods and treatments out of date – and infectious diseases are always in the news!

Point to consider 1.2: How many news stories involving microorganisms have you noticed during the last seven days? Find one article and think carefully about how well it was reported. Which points of information do you think were communicated well? Where there were inaccuracies, can you think of reason for the misunderstandings?

1.3 Techniques

Some techniques used to isolate and identify microorganisms have not changed substantially since they were first introduced – which in some cases is well over 100 years ago. This is usually because they are still useful and cost effective in helping to isolate and identify microorganisms from clinical samples. Others have been superseded by methods which give a more accurate or rapid result. It is therefore useful to critically evaluate techniques and to challenge assumptions about them. Does a traditional technique such as the Gram's stain still have a place in twenty-first century routine diagnostic microbiology? Why did virology services phase out virus isolation in monolayer cell culture? Is 'PCR' really the answer to every search for the optimal microbiology test?

The principles behind each technique will not be considered in any detail within this book. Readers are referred to laboratory protocols or standard operating procedures as appropriate, along with Wilkinson (2011) and Ford *et al.* (2014) for details.

Table 1.1 outlines some of the discussion points to consider about a selection of the techniques commonly used in diagnostic microbiology settings. The suite of tests used to investigate a particular clinical problem depends on a range of factors, which may include workload, available space, skill mix of workforce and service user requirements, as well as cost.

Point to consider 1.3: Select one of the techniques listed in Table 1.1 and think about how it is currently used in routine diagnostic microbiology (it might be helpful to focus on a type of infection, such as urinary tract infections, or a group of organisms, such as viruses). Do you think this method is used in the best way? Could the technique be improved? Would another type of test give better results?

1.4 Point-of-Care Testing (POCT)

There is a wide and expanding range of point-of-care tests (POCTs) for detection of microbial antigens or specific antibodies (Pitt, 2012; Moore, 2013; St John and Price, 2014). Many POCTs (also referred to as 'near patient tests' [NPTs] and 'rapid diagnostic tests' [RDTs]) utilise the lateral flow/immunochromatographic assay format. However, some use quite simple technology such as latex agglutination and others more complex principles including polymerase chain reaction (PCR) or other molecular methods (Moore, 2013). All are designed to be used outside of the main laboratory, by personnel who might not be laboratory staff – both healthcare professionals and in some cases, patients themselves. While it can be very useful clinically to get a rapid test result, there are a number of limitations of POCTs which scientists must be aware of (see Text Box 1.1).

It is important that senior laboratory scientific staff are involved in discussions about possible implementation of a POCT assay outside of the laboratory, such as on a hospital ward or in primary care. The main scientific issue with currently available POCT antigen detection tests is that most have relatively low sensitivities, although specificities are generally very good (Text Box 1.1; Moore, 2013). This means that the positive results can be trusted, but when the POCT does not detect the organism, follow-up laboratory tests are required to confirm the result. Maintenance of quality assurance is also a concern (Pitt, 2012; St John and Price, 2014) and the purpose of required procedures and documentation must be highlighted to those performing the test (which might include the patient). How the assay results are to be recorded must also be considered, in order that POCT test results are included in the patient's pathology profile (St John and Price, 2014). Some bench top analysers (e.g., PCR devices) keep an electronic record of the result, but this is not useful unless it can be connected to the main laboratory database. The other key factor in the decision of whether and how to use a POCT in microbiology is the cost per test (Pitt, 2012; St John and Price, 2014). While the price of kits themselves is usually relatively high, this has to be balanced against the staff costs (a senior scientist in the laboratory is more expensive per hour than a healthcare assistant on the ward) and time taken to make the diagnosis, implement treatment and discharge the patient. The proposed

Table 1.1 Comparison of selected techniques used in diagnostic microbiology departments to isolate and characterise microorganisms.

Technique	Use in diagnostic microbiology	Advantages	Disadvantages
Light microscopy (with appropriate stains to aid visualisation)	Detection of bacteria, fungi and parasites	Low cost Sample preparation straightforward and relatively quick Equipment easy to obtain and maintain 'Catch all' technique	Relatively low sensitivity Limit of resolution around 0.2 µm Recognition of organisms requires training and skill Slides examined individually so labour-intensive and time-consuming Follow-up tests usually necessary for full identification.
Light microscopy with fluorescent-labelled antibodies and UV microscope	Detection of bacteria, fungi and parasites	Very specific due to immunological detection method Good sensitivity as fluorescence easy to detect.	Expensive compared to use of general stain Detects pre-determined organisms only Slides examined individually so labour-intensive and time-consuming If no organism detected, further investigations may be indicated Requires ultraviolet (UV) microscope.
Culture on agar including selective, differential and chromogenic media	Isolation and initial identification of bacteria and fungi	'Catch-all' technique Selective media or specialised culture conditions exclude all but organism of interest to grow Chromogenic media allow presumption identification on basis of colonial appearance and colour change Live viable organism obtained to do further investigations –e.g., antimicrobial sensitivities, analysis of strain variation	Preparation of media time-consuming (most laboratories buy pre-prepared plates) Follow-up tests usually necessary for full identification.

Electron microscopy	Detection of viruses and small protozoa in clinical samples Detection/identification of viruses isolated in monolayer culture.	Limit of resolution is around 2 nm 'Catch all' technique Organisms identified due to morphological appearance.	Relatively low sensitivity Grids examined individually so labour-intensive and time-consuming Equipment very expensive to purchase and maintain Identification of structures requires specialised training and skill.
Monolayer cell culture	Detection of viruses (and some other obligate intracellular organisms) in clinical samples	'Catch-all' technique Detects viable viruses Live organism obtained to do further investigations –e.g., antiviral sensitivities, analysis of strain variation.	Takes days or weeks for viruses to grow and cells to show cytopathic effect (CPE) Requires specialised training and skill to interpret CPE Expensive Ethically questionable to use animal cells – especially primary cells.
Detection of antigen by enzyme immunoassay	Detection of bacterial, viral and protozoal antigens in clinical samples particularly blood and faeces	Good sensitivity and specificity Majority of assays provide result within a few hours Generic training sufficient to perform assay Can be automated to allow large batches of samples to be tested simultaneously.	Detects pre-determined organisms only If no organism detected, further investigations may be indicated Detects antigen rather than live viable organism Antigen may be genus-specific only Species-specific assays may not detect strain variation.
Detection of antibodies by enzyme immunoassay	Detection of specific antibody response to bacteria, viruses, fungi, protozoa and helminths in blood and other fluids	Good sensitivity and specificity Majority of assays provide result within a few hours Generic training sufficient to perform assay Can be automated to allow large batches of samples to be tested simultaneously Assays available to detect different classes of antibody which helps to indicate stage of infection.	Detects antibody to pre-determined organisms only If no specific antibody detected, further investigations may be indicated Detects evidence of presence of organism rather than live viable organism.

(*Continued*)

Table 1.1 (Continued)

Technique	Use in diagnostic microbiology	Advantages	Disadvantages
Polymerase chain reaction	Detection of specific DNA or RNA sequence characteristic of virus, bacterium, fungus, protozoan or helminth in clinical samples	Very specific and sensitive Majority of assays provide result within one working day Generic, albeit specialised, training sufficient to perform assay Can be automated to allow batches of samples to be tested simultaneously Multiplex assays allow assay for suite of organisms in single test PCR product can be sequenced to allow further characterisation.	Detects genome of pre-determined organisms or set of organisms only If no genome detected, further investigations may be indicated Detects genome of organism rather than live viable organism Relatively expensive Requires specialised equipment, several preparation and analytic steps and separate rooms to minimise contamination.
Matrix-assisted laser desorption/ ionisation – time of flight (MALDI-TOF)	Identification of bacteria and fungi from isolates	Specific and good sensitivity Provides result quickly and easily in comparison to a full set of biochemical tests Relatively cheap Protein sequence data can indicate antimicrobial sensitivities.	Requires comprehensive database – may not recognise new or unusual organisms.

Text Box 1.1 Advantages and Limitations of using Point-of-Care Tests for Infectious Diseases

Advantages

Provide rapid results; allows quick decision about management.
Require small amount of relatively easy to collect sample.
Easy to use; can be performed by anyone who has been given suitable training (and by following SOP instructions).
Kits often contain all required reagents and equipment.
Specificity usually very good (up to 100%).

Limitations

Expensive compared to standard laboratory assays.
Any excess sample discarded; follow-up tests require fresh collection.
Quality assurance can be overridden/overlooked.
Sensitivity usually relatively low (50% in some cases).

role of the POCT test in the patient care pathway must therefore be considered carefully (Moore, 2013; St John and Price, 2014).

Because they are quick and easy to carry out, microbiology POCTs are also widely used as part of main laboratories' repertoires, as initial or confirmatory tests – in spite of their limitations. In this situation, the information from the POCT can be put with other results. Outside of the laboratory, the most obvious role for POCT tests is where a wait for the results might compromise patient care. Examples of this type of situation include an outbreak of an infection (e.g., norovirus) where diagnosis is important for the individual, but is also necessary to minimise spread by isolation and cohort nursing of affected patients (Moore, 2013). Genitourinary medicine clinics have found POCT tests useful in situations where patients might not be prepared to wait or return at a later time for a result, for instance for a syphilis test (Pitt, 2012). They also have a role where the main laboratory is not easily accessible, such as the bench top device for measuring blood CD4+ counts in HIV patents living in remote areas (St John and Price, 2014). Use of a *Plasmodium* RDT in rural clinics in endemic countries (instead of relying solely on a clinical picture of pyrexia and rigors), should reduce misdiagnosis of malaria. Erroneous presumption of *Plasmodium* infections can lead to over use of anti-malarial drugs and the potential lack of appropriate treatment for other conditions (Pitt, 2012). Another interesting way in which microbiology POCTs have been used is in 'satellite' laboratories, within a multi-site healthcare organisation (e.g., Cohen-Bacrie *et al*., 2011). The idea is to provide results at each hospital site where possible, to avoid the costs, logistical difficulties and risk of loss of integrity of the specimen, inherent in transport between locations. In this case laboratory staff are performing the tests, so they should have good insight into the limitations and also immediate access to support from the main laboratory (in terms of advice and the option of sending samples for further tests) if necessary. For more in depth consideration of the range and potential applications of POCT within diagnostic services, the reader is referred to other sources, such as Price and St John (2012).

Point to consider 1.4: How do you think microbiology POCTs could be used to best effect?

1.5 Antimicrobials

The advent of antimicrobial agents during the twentieth century drastically changed people's attitudes to infection. The list of feared, commonly fatal diseases changed from including infections including scarlet fever, gangrene from infected wounds, tuberculosis and syphilis, to various type of cancer. The course of non–life-threatening infections can also be shortened, which reduces the number of days the individual loses to illness, as well as decreasing the risk of secondary infections (Davey *et al*., 2015). Since antibiotics are generally considered as safe drugs to take and courses of treatment are time-limited, the expectation by patients that they can be taken to treat even mild infections has arisen. Widespread overuse and misuse of the drugs have created the niches that microorganisms needed to develop resistance and this is now considered a major threat to public health (e.g., Shallcross and Davies, 2014). In response, guidelines are being issued for prescribing, which encourage more careful deliberation of the clinical need (e.g., NICE NG15, 2015). The microbiology laboratory scientist should have an understanding of the range of antimicrobial agents, their mechanisms of action (including selective activity) and the nature of mutations leading to resistance. This is to help inform decisions about antimicrobial testing protocols within the laboratory and also to understand how to contribute to surveillance for reduced susceptibilities. An overview of antibiotics, antivirals and antifungals is given below, while the treatment and management of the infections included in this book are addressed in the relevant chapters. Detailed discussion of antimicrobial activity, resistance mechanisms and treatment regimes is beyond the scope of this book and the reader is referred to Davey *et al*. (2015) – or other suitable texts – for in depth consideration of this subject.

1.5.1 Antibacterial Agents (Antibiotics)

As Figure 1.1 indicates, there is a range of potential weak points in the bacterial life cycle which could be targets for antibacterial agents. The differences in structure and metabolism between prokaryotic and eukaryotic cells mean that the effects can be quite specific, with low toxicity for the mammalian host. Table 1.2 summarises the mode of action and mechanism(s) of resistance for selected antibacterial drugs. Some antibacterial agents also work against other organisms. Examples include co-trimoxazole (trimethoprim plus sulphamethoxazole) which is used to treat *Pneumocystis jirovecii* pneumonia and metronidazole is often the drug of choice for infections with the gut protozoa *Entamoeba histolytica* and *Giardia duodenalis*.

1.5.2 Antiviral Agents

There are relatively few effective antiviral agents and they usually have limited and very specific uses. This is partly due to the lack of opportunities for selective toxicity – since viruses are reproducing inside host cells it can be hard to find a target peculiar to the virus. The other problem is the speed at which resistant virus strains can arise, particularly in RNA viruses. The first safe, effective and widely used antiviral was aciclovir (Zovirax®). It was a discarded candidate anti-cancer drug which was found to have activity against herpes simplex and varicella zoster viruses (Elion, 1983). It transpired

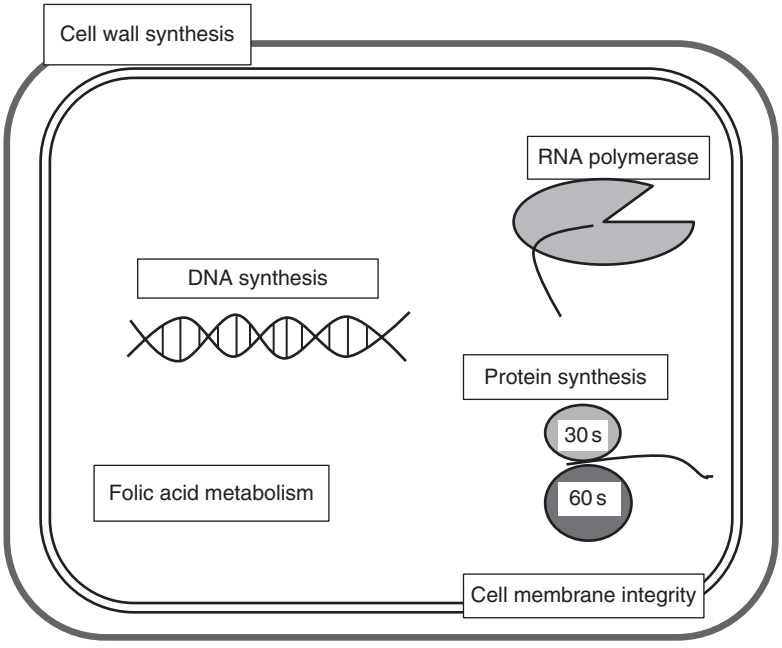

Figure 1.1 Outline of generic bacterial cell indicating possible targets for antibacterial agents.

that aciclovir is a substrate readily taken up by the viral thymidine kinase in purine metabolism (as outlined in Figure 1.2), but which does not have affinity for the equivalent host cell thymidine kinase. Subsequent progress in finding similarly specific 'viral enzyme-substrate' reactions in other virus species has been slow. Table 1.3 outlines some details about the main antivirals currently available.

1.5.3 Antifungal Agents

As fungi are eukaryotes, there is a restricted range of metabolic pathways which are not shared with mammalian host cells. Thus, most anti-fungal agents are rather toxic to patients and topical preparations are used wherever possible. The first antifungal to be introduced was griseofulvin (Davey *et al.,* 2015). It is thought to work by affecting mitosis in the dividing fungal cell. However, it is only effective when given orally and only works against dermatophytes. A key target area for anti-fungals is the sterols in the fungal cell membrane. Polyenes (e.g., amphotericin, nystatin) bind to sterols, thus disrupting the structure of the membrane. These are unfortunately not selectively toxic and only amphotericin is safe enough to give systemically (with careful monitoring!). Ergosterols are an essential membrane component found in fungal, but not mammalian membranes. Azoles (imidazoles and triazoles) inhibit the activity of lanosterol demethylase, which catalyses the conversion of lanosterol to ergosterol within the fungal cell. Examples include clotrimazole (an imidazole) and fluconazole (a triazole). Azoles are hepatotoxic in humans, so external treatment is used wherever feasible. Triazoles are used in preference to imidazoles in oral treatment regimens as they have more suitable pharmacological properties, but their use is reserved for severely debilitating or

Table 1.2 Site and mode of action and mechanism of resistance for selected antibiotics.*

Site of action	Antibiotic class (examples)	Mode of action	Mechanism of resistance	Susceptible organisms[1]
Cell wall synthesis	β-lactams (pencillins, cephalosporins)	Disruption of peptidoglycan cross linking; Inhibition of cell wall formation by attaching to penicillin binding proteins.	Production of β-lactamase; Modification of penicillin-binding proteins; Alteration of membrane permeability.	
	Glycopeptides (vancomycin, teicoplanin)	Inhibition of cell wall building through binding to acyl–D-alanyl-D-alanine	Substitution of alanine for lactate or serine	
Protein synthesis	Aminoglycosides (streptomycin, gentamycin, tobramycin)	Binding to ribosomes to interrupt formation of mRNA	Productions of enzymes which alter aminoglycoside structure or modifications to membrane proteins thus inhibiting transport into bacterial cell	
	Chloramphenicol	Obstruction of peptidyl-transferase reaction on ribosome	Production of chloramphenicol acetyltransferase to modify molecule and alteration in protein sequence of 50S subunit to prevent binding to ribosome; Change in membrane structure to inhibit transport into bacterial cell	
	Tetracyclines (tetracycline, doxycyline, tigecycline)	Binding to 30S subunit, preventing attachment of t-RNAs	Production of membrane protein associated with rapid removal of antibiotic from bacterial cell; Production of protein in cytoplasm which prevents binding to ribosome	
	Macrolides (erythromycin, clarithromycin)	Removal of nascent peptide chain from ribosome	Production of enzyme which alters structure of 23S subunit by adding methyl group	

Nucleic acid synthesis	Sulphonamides	Competitive substrate against PABA for dihydropteroate synthetase in folic acid pathways thus preventing production of purines	Production of alternative dihydropteroate synthetase which sulphonamides cannot attach to
	Trimethorprims	Competitive substrate against dihydrofolic acid for dihydrofolic acid reductase in folic acid pathways thus preventing production of pyrimidines	Production of alternative dihydrofolic acid reductase which sulphonamides cannot attach to
	5-Nitroimidazoles (metronidazole)	Not fully understood; possibly integration into DNA	Not fully understood
	Rifampycins (rifampicin)	Binding to β-subunit of DNA–dependent RNA polymerase	Modification to β-subunit
Membrane function	Polymixins (colistin)	Disruption of cell membrane integrity	Limited information since drug not widely used (has same effect on mammalian cell membrane)

*Information taken from Davey *et al.* (2015); see this reference for further details particularly Chapters 2,3,4 and 11.
[1] The final column has been left blank for the reader to fill in. See Exercise 1.1 below.

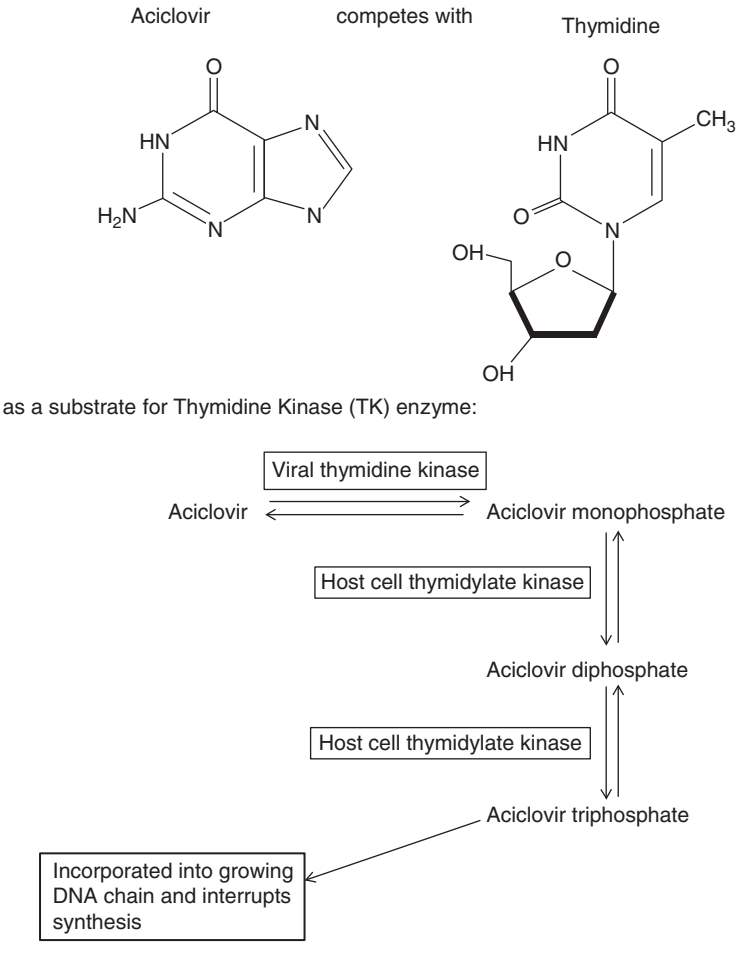

Figure 1.2 Mode of action of aciclovir.

life threatening infections (Davey *et al.,* 2015). Flucytosine is an example of a nucleotide analogue; it is metabolised within the fungal cell to 5-fluorouracil which is taken up by RNA polymerase, thus interrupting production of RNA. Unfortunately, this reaction only occurs in yeasts.

1.5.4 Antiparasitic Agents

Protozoa and helminths are also eukaryotic and they are relatively complex compared to other microorganisms. They tend to have multiple life cycle stages and most species use sexual as well as asexual replication mechanisms. This means that the similarities with mammalian cells are greater than for bacteria and viruses. Therefore the range and scope of safe, efficacious anti-parasitic agents is limited (Davey *et al.*, 2015). Long-standing treatments, such as quinine/chloroquine for malaria and arsenical compounds for trypanosomiasis are still used in spite of associated problems. Chloroquine resistant strains of

Table 1.3 Mode of action and selective toxicity for selected antiviral agents.*

Antiviral drug	Virus(es) it is effective against	Mode of action	Selective toxicity
Aciclovir (ACV)	Herpes simplex; varicella zoster	Analogue of guanosine; phosphorylated inside cell to be substrate for thymidine kinase in nucleoside-TP production; ACV-TP interrupts DNA replication by causing chain termination and inhibiting DNA polymerase	Phosphorylation only virus-infected cells ACV is a substrate only for viral thymidine kinase; cannot be activated by host enzyme
Ganciclovir (GCV)	Cytomegalovirus	Analogue of guanosine; phosphorylated inside cell by phosphotransferase enzyme; GCV-TP interrupts DNA replication by causing chain termination and inhibiting DNA polymerase	Not selectively toxic – GCV also substrate for host phosphorylation enzymes
Ribavirin	Respiratory syncytial virus Hepatitis C virus	Nucleoside analogue; phosphorylated inside cell. Exact effects unclear, presumed to be different in RNA and DNA viruses	Substrate for viral enzymes; however can affect host cells (e.g., causing haemolytic anaemia)
Foscarnet	Cytomegalovirus	Pyrophosphate analogue; direct DNA polymerase inhibitor	Substrate for viral enzymes; however can affect host cells (e.g., kidney cells causing acute renal failure)
Zidovudine (AZT)	Human immunodeficiency virus	Nucleoside analogue; activated to triphosphate form by host enzymes; AZT-TP causes chain termination in DNA replication and inhibits reverse transcriptase	Not selectively toxic – AZT activated by host enzymes; toxic to fast metabolising cells such as in bone marrow
Lamivudine	Hepatitis B virus	Nucleoside analogue; activated to triphosphate form by host enzymes; Lamivudine-TP causes chain termination in DNA replication and inhibits DNA polymerase	Substrate for viral enzymes
Tenofovir	Hepatitis B virus	Analogue of adenine mono-phosphate; inhibits DNA replication	Substrate for viral enzymes
Efavirenz	Human immuno-deficiency virus	Bind to reverse transcriptase and inhibits its activity	Substrate for viral enzymes
Oseltamivir	Influenza virus A and B	Blocks virus entry into host cell by inhibiting neuraminidase	Binds to specific viral protein
Maraviroc	Human Immuno-deficiency virus	Blocks virus entry by inhibiting binding to CCR5 receptor on host cell	Blocks virus-specific activity
Amantidine	Influenza A virus	Blocking uncoating of virus particle within host cell	Binds to specific viral protein; can affect neurone function causing insomnia and confusion

*Information taken from Davey *et al*. (2015); see this reference for further details, particularly Chapters 5, 6 and 7.

Plasmodium spp., particularly *P. falciparum* are widespread. While melarsoprol, which is an arsenic-based drug kills *Trypanosoma brucei* spp., it can also kill the host! Amphotericin is effective against *Leishmania* spp., but as mentioned above it is toxic to mammalian cell membranes as well. There are a number of anti-*Plasmodium* drugs (Davey *et al*., 2015), although resistance is constant problem in malaria. As mentioned above, metronidazole is effective treatment for the gut protozoa *E. histolytica* and *G. duodenalis*. It works by disrupting DNA synthesis, although the mode of action has not yet been fully elucidated. Although there are only a few anthelmintic agents (note the spelling – a shortened version of 'anti-helminthic', which is much easier to say), they each tend to have a broad spectrum. Albendazole and mebendazole (benzimidazoles) are used to treat nematodes such as hookworm and *Ascaris lumbricoides*. They are thought to interrupt several points in the worm's metabolism including formation of microtubules in the cytoskeleton and production of ATP. The worm is weakened and eventually unable to move. Praziquantel is an effective treatment for flukes, such as *Schistosoma* spp. (but not *Fasciola hepatica*) and tapeworm infections (Davey *et al*., 2015). It is thought to work by altering the calcium balance across the cells, similarly resulting in paralysis. It also disrupts the tegument (outer coat) which exposes the helminth's antigens to the host's immune system. Ivermectin also affects the neuromuscular system and can be used against ectoparasites such as the scabies mite (*Sarcoptes scabei*) as well as some parasitic worms (Gunn and Pitt, 2012). All these anthelmintic drugs can be toxic to humans, but often only one or two doses are required to fully clear the infection.

Point to consider 1.5: How could more detailed knowledge of the genetics and metabolism of viruses and funguses help in the search for more selectively toxic agents?

1.5.5 Antimicrobial Sensitivity Testing

Testing for antimicrobial sensitivities accounts for a sizeable amount of the workload in diagnostic microbiology departments. The importance of doing this is one of the arguments in favour of retaining laboratory techniques which allow isolation of viable organisms from patient samples. For bacterial and fungal susceptibility testing, laboratories in the UK should follow the guidelines issued by the British Society for Antimicrobial Chemotherapy (BSAC). This organisation sets standards for laboratory methods and conducts surveillance of resistance for clinically important bacteria and fungi. It uses the information collected to provide tables of breakpoints for each organism against all routinely prescribed antibiotics (www.bsac.org.uk). Since the issues related to antimicrobial resistance are international, BSAC works closely with similar organisations in other parts of the world, notably in Europe. In order to coordinate resistance data more effectively, UK clinical microbiology laboratories phased in the change to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) protocols and breakpoint data, from January 2016 (www.bsac.org.uk). This means using a standardised disc diffusion assay (based on the Kirby-Bauer method and similar to the BSAC method) for antibacterial susceptibilities and a broth microdilution method to determine minimum inhibitory concentrations (www.eucast.org). A microdilution method is also recommended for susceptibility testing of isolates of yeasts, with a tube dilution method promoted for filamentous fungi (www.eucast.org). The EUCAST testing guidelines and

surveillance remit also extends to organisms of veterinary importance (www.eucast.org). For a review of EUCAST and decision-making systems to determine and monitor antibiotic resistance see Winstanley and Courvalin (2014).

As matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) becomes more widely used for routine characterisation of microorganisms, the power of the database in distinguishing between strains within species is increasing. This means that the MALDI-TOF profile of a clinical isolate has the potential to be used not only in full identification, but also in predicting its antimicrobial susceptibilities (Weiser *et al*., 2012). There are clear advantages to this from a patient management point of view, particularly in situations where waiting an additional 24 hours for the antibiotic results on a new isolate may be unacceptably long. Although the MALDI-TOF result is based on a protein profile and not biological activity, it does appear to allow the identification of some antibiotic resistance profiles (Weiser *et al*., 2012). Nevertheless, conventional testing methods will continue to have an important role in monitoring newly arising resistance (i.e., resistance gene spread to new species or new mutations affording mechanisms of resistance).

In contrast, as routine virus detection has moved to molecular methods, viral susceptibility testing has largely followed suit and is usually based on genome sequencing (Jeffery and Aarons, 2009). Resistance to a particular antiviral is associated with one or more specific genetic mutations, which can be ascertained by comparing the sequence to a database. Phenotypic assays for antivirals and monitoring of (live) isolates is still undertaken in reference laboratories and this helps to keep the reference databases current. Since the available treatment options for parasite infections are limited, susceptibility testing is not usually undertaken except in reference laboratories and research centres.

Point to consider 1.6: Surveillance of antimicrobial resistance is conducted at national and international level. Chose a specific example and think about how effective has this been in reducing the spread of resistance in the last five years.

1.6 Selection and Evaluation of Diagnostic Tests

The logistical and financial factors which are currently driving the configuration of pathology services can pose interesting challenges. One of these is combining the available equipment and expertise across two or more geographically separated sites, which had hitherto operated independently. The laboratory staff in this situation often find themselves navigating their way through a series of apparently conflicting issues and interests, but scientific and clinical considerations must be central in decision making (Price, 2012). Other points to consider include financial and logistical – for example, whether a complex assay in the main laboratory could be complemented (or replaced entirely!) by a point-of-care test (Cohen-Bacrie *et al*., 2011) – and also which tasks can appropriately be assigned to which grade of staff. Dialogue with the service users is also important to ensure that the results provided by an assay are appropriate to the clinical need (Price, 2012; Price and Christensen, 2013). Expenditure can be maintained at manageable levels by reducing repetition of tests (e.g., the same test requested by the GP and then at the hospital a few hours later) and ensuring that the assay still detects the most relevant marker (Price and Christensen, 2013).

The postgraduate level laboratory scientist should be aware of the context in which the pathology service is operating in order to contribute effectively to decisions made by service managers. The concept of 'evidence-based laboratory medicine' (EBLM) as developed by Price and Christensen (Price *et al.,* 2009) is a useful approach. They describe a five-point cycle:

1) Ask the right question
2) Acquire suitable evidence
3) Appraise the information
4) Apply the outcome
5) Audit the service provided (Price, 2012).

Whether the laboratory team is thinking about changing the configuration of a service, replacing one assay with another for a particular marker or introducing a novel test, Price and colleagues suggest that applying EBLM can help. For detailed consideration of the topic, the reader is referred to Price *et al*. (2009) or Price (2012), but it is worth noting that the emphasis is on applying scientific understanding and ways of thinking to improving the overall outcome for the patient (Price, 2012). The complexity of the situation is highlighted by their discussion of the first step in the cycle – asking the right question. There are a range of people with different perspectives all asking questions relating to the service and they each need to be included in the analyses (Price and Christenson, 2013). To illustrate this, take the example of the possible introduction of a POCT test for norovirus to be used on the wards to enhance rapid diagnosis in patients with gastroenteritis. The patient will be wondering what type of sample is to be used, whether it will be painful and uncomfortable to collect, how long will it take for the result to be ready and what the result could mean for them. Is it a simple stool sample? Will the result be ready on the same day? The staff on the wards will be thinking about how easy it is to perform the test, how much training might be required before being able to carry it out, where they could perform the assay, as well as turnaround time and interpretation of the results. Does the test come in a kit with straightforward instructions? If the result is positive does that mean that the patient definitely does have norovirus and should be nursed separately? The laboratory scientist will be thinking about technical considerations including comparative sensitivity and specificity of the POCT method, effective training of ward staff to carry out the test and quality control procedures. Is the test sensitive enough to pick up all infections even in patients presenting quite early (or late)? Is it specific enough that negative results can be trusted? How easy is it to ignore some part of the instructions and still obtain a result with this particular kit format? There will also be management issues such as overall cost, implications for the relevant laboratory and ward staff workloads, planning and commissioning of services, infection control and reliability of data used for surveillance. The laboratory manager will want to know whether the cost of the POCT kits will come out of the pathology or the ward budget, but also how many samples might have to be re-tested in the main laboratory (if it is above a certain level, the POCT test might not be suitable). If there is a regional or national outbreak of norovirus, the local commissioners and or government departments might want to put more resources into diagnosis. Again, they will need to know the overall cost per test (which includes staff time, as well as the price of

Text Box 1.2 Requirements of an Assay Used in Diagnostic Pathology

Tests for a clinically useful marker (whole organism, genome, antigen, antibody or other marker).
Gives reliable results – that is, scientifically accurate and clinically credible.
Results are reproducible – that is, testing aliquots of the same sample repeatedly will give the same results each time.
Assay has high sensitivity and specificity (see Text Box 1.3).
The turnaround time for the result is appropriate to the clinical condition.
The method is straightforward for the operator after suitable training has been undertaken.
The assay uses equipment and kits (or reagents) which are readily available from a reliable supplier.
The test procedure is cost-effective overall.

equipment and consumables) and time taken to obtain a reliable result – which affects patient management. It is important to appreciate all these points of view to inform decision making (Price and Christenson, 2013). Evidence should be collected from consultation with service users, systematic review of the literature and discussions with colleagues in other laboratories; the outcome of the appraisal can then be used in a report or business case for presentation to managers or funding bodies (Price, 2012).

When the evidence suggests that a particular test should be evaluated, the laboratory-based scientist usually begins with an overview of its suitability. Text Box 1.2 outlines the characteristics required of a diagnostic test in pathology. It should be noted that it can be a false economy (and unhelpful to the patient) to prioritise cost per test and turnaround time at the cost of accuracy of the result. A test with a low specificity will produce false positives, which will need repeat analysis, and probably re-testing in a confirmatory assay. If fresh samples are required for this follow-up work, then the patient may also be needlessly distressed.

For the technical part of the assessment, the 'gold standard' method for that marker has to be identified. This is usually the method that is generally accepted to be the most sensitive and specific, reliable and accurate. Sometimes it is not feasible to use this (e.g., where the gold standard is only carried out in reference centres) and in that case, the assay currently used by the laboratory can be designated as the gold standard for the calculations. A well-designed study should be implemented involving a predetermined number of samples, with appropriate inclusion criteria and suitable controls. The samples should be run in the new assay and the gold standard in parallel and the results compared. As a reminder, the equations used to calculate the sensitivity, specificity and positive and negative predictive value (PPV and NPV) are shown in Text Box 1.3. It is important to note that in a population where the prevalence of a diagnostic marker is low, the PPV can be significantly reduced. In a diagnostic situation this may not be a prime consideration, but it should be borne in mind when implementing screening programmes (e.g., testing of asymptomatic patients for HIV antigen/antibody in a low-risk population in Western Europe).

Text Box 1.3 Comparison of an Assay With the 'Gold Standard'

1) Set out the data in the following format:

	Result in gold standard	assay
Result in new assay	Positive	Negative
Positive	True positive	False positive
Negative	False negative	True negative

2) Calculate sensitivity and specificity:

$$\text{Sensitivity} = \frac{\text{true positives}}{\text{true positives} + \text{false negatives}} \times 100 = \% \text{ sensitivity}$$

$$\text{Specificity} = \frac{\text{true negatives}}{\text{true negatives} + \text{false positives}} \times 100 = \% \text{ specificity}$$

3) Calculate positive and negative predictive values:

$$\text{PPV} = \frac{\text{true positives}}{\text{true positives} + \text{false positives}} \times 100 = \% \text{ PPV}$$

$$\text{NPV} = \frac{\text{true negatives}}{\text{true negatives} + \text{false negatives}} \times 100 = \% \text{ NPV}$$

1.7 Quality Management

It is clear that the overall quality of a clinical microbiology service is dependent on a range of scientific, technical, managerial and human factors (Pitt and Sands, 2002). The laboratory scientist must be aware of and fully engaged with all the recommended internal procedures for quality assurance and all the external quality assessment and accreditation requirements. It is assumed that each reader will be introduced to this as appropriate for their job role, but sources of useful information include: Microbiology UKNEQAS for Microbiology (www.ukneqasmicro.org.uk) Quality Control for Molecular Diagnostics (www.qcmd.org), United Kingdom Accreditation Service (www.ukas.com) and the European Society of Clinical Microbiology and Infectious Diseases (www.escmid.org). There are also textbooks available to provide guidance on preparing for accreditation, such as Burnett (2013).

1.8 Infection Control, Monitoring and Surveillance

It is useful for the scientist to appreciate the laboratory's role in infection control and outbreak investigation at a local level. It contributes to identifying cases and possible sources of infection, allowing measures to be put in place to manage individuals and reduce spread of disease. Recognising and reporting notifiable diseases is also

imperative, as that information feeds into surveillance mechanisms at regional, national and international level. Monitoring of genetic variation within species is also a key role (although this is often carried out in specialist centres and reference laboratories) as this can lead to recognition of factors which might account for increased virulence or antimicrobial resistance. A number of techniques are proving valuable in this respect for monitoring outbreaks of microbial infections (e.g., Sabat *et al.*, 2013) and these include 'whole genome sequencing' (e.g., Halachev *et al.*, 2014) and the high throughput 'next-generation (or 'deep') sequencing' (e.g., Sherry *et al.*, 2013; Quiñones-Mateu *et al.*, 2014).

An 'outbreak' of infection is an epidemiological concept which is defined by precise criteria:

1) A situation where at least two people who can be connected – due to contact with each other or visiting a particular place at the same time – are exhibiting similar symptoms; or
2) The incidence of a particular infection is higher than usually recorded in that geographical area, at that time of year and in that population; or
3) One reported case of an uncommon infection such as diphtheria, rabies or poliomyelitis; or
4) Known or expected contamination of a food or water source.

When these criteria are met, this allows for an outbreak control team to be convened to manage the situation (McAuslane *et al.*, 2014). Laboratory diagnosis of suspected cases is not necessarily required to trigger the outbreak procedure, but it is needed to move the individual case definition to 'confirmed'.

Investigations of outbreaks of gastrointestinal disease such as norovirus infection or food poisoning can be challenging, since in healthy adults, they are usually mild and self-limiting. People often disperse after being in contact with the source and may not make the connection between being in a particular building or eating a certain meal and their symptoms of diarrhoea and vomiting. If they do not seek medical help, their illness will not be recorded. It may be days or weeks before there is sufficient indication of an outbreak. Identifying cases can be difficult, because people tend to shed viable organisms for very limited time periods after recovery. In addition, examination of food items is rarely possible, as they have usually been discarded. Therefore reviews of such events rely heavily on gathering of epidemiological evidence and deciding the most likely source and probable scenario of spread. For example Edwards *et al.* (2014) reported an outbreak of *Campylobacter* food poisoning, which was associated with the consumption of chicken liver pâté at a wedding reception. Someone reported that a number of people had become ill after being at the wedding. Once a pattern had been noticed, enquiries into potential contacts (i.e., wedding guests) yielded a list of 118 people. These were sent a questionnaire to ascertain various details including how long they were present at the reception and what they ate (this was important as two separate meals were provided by caterers during the course of the day). 'Cases' were defined as those who had eaten at the reception and who experienced diarrhoea or vomiting within 5 days (Edwards *et al.*, 2014). Those who had been abroad immediately before the wedding and anyone whose household contacts had diarrhoea symptoms, but had not been at the event, were excluded from the study. One hundred and eight of those approached returned

a questionnaire and of those 49 met the criteria to be categorised as a case. Interestingly, 31 people had sought medical advice for gastrointestinal symptoms, but faecal samples were only obtained for 26 of them. *Campylobacter* infection was confirmed in 22 people, but it was not possible to obtain any food for testing. Examination of the data collected suggested that the common factor amongst all those affected was consumption of the chicken liver pâté (Edwards *et al.*, 2014). Discussions with the food preparation staff indicated that they had not followed the Food Standards Agency's guidelines about cooking chicken liver designed to kill *Campylobacter* spp. and thus it was concluded that this bacteria was the causative agent. It is worth noting that the reason that the investigation was begun was because one person knew several of the wedding guests; when people at an event do not know each other very well, that initial connection is often not made. Also, in this case a reasonable number of people were sufficiently worried about their symptoms to see a doctor and stool samples were collected from most of those at the time of presentation. This may not happen and it is therefore sometimes hard to identify those who were infected but whose symptoms did not trouble them much at the time; where few people submit samples and there is no food or fomite from the presumed source, it may not be possible to find the cause at all.

Surveillance is important as part of general public health measures, thus obtaining data to fulfil the 'outbreak' criteria number 2 and number 3 in the list above. Laboratory support, to confirm suspected cases, is a very valuable part of this. Data reported to central agencies at national level can be collated to investigate actual or possible links between outbreaks. For example, outbreaks of measles can occur in areas where MMR coverage is sub-optimal. In the UK, laboratory confirmed cases of measles are notifiable, which allows connections between individuals to be noticed and for healthcare professionals to be alerted. There was an unexpectedly high incidence of measles in England and Wales in 2012. The majority of cases were recorded in either the South East (Sussex) or the northwest (Merseyside) of England, so the infection appeared to be contained within limited geographical pockets. Then at the end of 2012, a very significant outbreak covering the whole of Wales began (Pitt, 2013); this highlighted the value of surveillance data and the importance of vigilance in control of infectious diseases, as well as providing a lot of work for the virologists! The incidence of measles in Scotland and Northern Ireland did not increase during this time, which is probably attributable to better MMR vaccination rates in those two countries (Pitt, 2013). Similarly, while the spread of West Nile Virus across the United States between 1999 and 2008 was rapid and alarming (https://www.cdc.gov/westnile), surveillance data did contribute to understanding the epidemiology of the infection and thus to bringing it under control (Lindsey *et al.*, 2014).

Member states in the European Union (EU) and European Economic Area (EEA) must report confirmed cases of certain infectious diseases to the European Centre for Disease Prevention and Control (ECDC: http://ecdc.europa.eu); this allows trends across the continent to be noted. For instance, in the summer and early autumn of 2012, Germany, the Netherlands and the UK all reported increased incidence of *Cryptosporidium* infection. An investigation into possible common factors and an assessment of Europe-wide risks (ECDC, 2012) concluded that there was no common source of the parasite even within each country and that the likelihood of similar occurrences across a wider area of the continent was low (ECDC, 2012). However, it was useful to highlight the issue, to alert microbiologists and public health professionals in

other countries to consider cryptosporidiosis as a diagnosis for people who had recently travelled to any of the three countries. From a wider perspective, the World Health Organisation (www.who.int) also collects and analyses data from 194 countries, using its six regional offices to coordinate responses to epidemics and pandemics and work to improve control and prevention of infection. An example is the activity by the WHO European Region to understand and contain Middle Eastern Respiratory Syndrome Coronavirus, MERS-CoV (http://www.euro.who.int/en/health-topics/communicable-diseases/influenza/middle-east-respiratory-syndrome-coronavirus-mers-cov/mers-cov-in-the-who-european-region).

1.9 Exercises

1.1 Find examples for the final column in Table 1.2 – labelled 'susceptible organisms'.

In each case think about:

- **A** How many examples of susceptible organisms are available for antibiotics in that group and the implications of this in terms of spread of resistance
- **B** The clinical application and continued usefulness of each group of antibiotics

1.2 Chose a protocol used for either the investigation of a clinical condition (e.g., UTI) or diagnosis of a specific pathogen (e.g., herpes simplex virus) in your laboratory or from the published literature.

- **A** Think about which techniques are used and why they might have been selected.
- **B** Do you think that this protocol is the optimal method?
- **C** Are there any ways in which it could be improved to enhance microbiology service delivery?

1.3 Select a microbiology POCT assay currently on the market.

- **A** Search the literature to investigate research its technical characteristics, how well it has been assessed and how it is used in diagnostic microbiology.
- **B** Design an evaluation study which would help to decide whether to use this test in a *specific* clinical context (e.g., paediatric ward, primary care).
- **C** Prepare the outline of a short oral presentation or written report in which you would present your findings to a group of clinicians and managers who would decide whether this POCT should be implemented.

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