Vaccines

LEARNING OBJECTIVES

18-1 Define *vaccine.*

18-2 Explain why vaccination works.

18-3 Differentiate the following, and provide an example of each: attenuated, inactivated, toxoid, subunit, and conjugated vaccines.

18-4 Contrast subunit vaccines and nucleic acid vaccines.

18-5 Compare and contrast the production of whole-agent vaccines, recombinant vaccines, and DNA vaccines.

18-6 Define *adjuvant.*

18-7 Explain the value of vaccines, and discuss acceptable risks for vaccines.

Long before the invention of vaccines, it was known that people who recovered from certain diseases, such as smallpox, were immune to the disease thereafter. Chinese physicians may have been the first to try to exploit this phenomenon to prevent disease when they had children inhale dried smallpox scabs.

In 1717, Lady Mary Montagu reported from her travels in Turkey that an "old woman comes with a nutshell full of the matter of the best sort of smallpox and asks what veins you please to have opened, and puts into the vein as much venom as can lie upon the head of her needle." This practice usually led to a week of mild illness, and the person was subsequently protected from smallpox. Called **variolation,** this procedure became commonplace in England. Unfortunately, however, it sometimes resulted in a serious case of smallpox. In eighteenth-century England, the mortality rate associated with variolation was about 1%, still a significant improvement over the 50% mortality rate that could be expected from smallpox.

One person who received this treatment, at the age of 8, was Edward Jenner. As a physician, Jenner subsequently encountered patients who did not respond with the usual reactions to variolation. Many of them, especially dairymaids, told him that they had no fear of smallpox because they had already had cowpox. Cowpox is a mild disease that causes lesions on cows' udders; dairymaids' hands often became infected during milking. Motivated by his childhood memory of variolation, Jenner began a series of experiments in 1798 in which he deliberately inoculated people with cowpox in an attempt to prevent smallpox. To honor Jenner's work, the term *vaccination* (from the Latin *vacca*, meaning cow) was coined. A **vaccine** is a suspension of organisms or fractions of organisms that is used to induce immunity. Two centuries later, the disease of smallpox has been eliminated worldwide by vaccination, and two other viral diseases, measles and polio, are also targeted for elimination. See the box on page 505. **Animation** Vaccines: Function. **www.microbiologyplace.com**

CHECK YOUR UNDERSTANDING

✓ What is the etymology (origin) of the word *vaccine*? **18-1**

Principles and Effects of Vaccination

We now know that Jenner's inoculations worked because the cowpox virus, which is not a serious pathogen, is closely related to the smallpox virus. The injection, by skin scratches, provoked a primary immune response in the recipients, leading to the formation of antibodies and long-term memory cells. Later, when the recipient encountered the smallpox virus, the memory cells were stimulated, producing a rapid, intense secondary immune response (see Figure 17.16, page 494). This response mimics the immunity gained by recovering from the disease. The cowpox vaccine was soon replaced by a vaccinia virus vaccine. The vaccinia virus also confers immunity to smallpox, although, strangely, little is known with certainty about the origin of this important virus. It is genetically distinct from cowpox virus and may be a hybrid of an accidental mixing of cowpox and smallpox viruses or perhaps once may have been the cause of a now-extinct disease, horsepox. The development of vaccines based on the model of the smallpox vaccine is the single most important application of immunology.

Many communicable diseases can be controlled by behavioral and environmental methods. For example, proper sanitation can prevent the spread of cholera, and the use of condoms can slow the spread of sexually transmitted infections. If prevention fails, bacterial diseases can often be treated with antibiotics. Viral diseases, however, often cannot be effectively treated once contracted. Therefore, vaccination is frequently the only feasible method of controlling viral disease. Controlling a disease does not necessarily require that everyone be immune to it. If most of the population is immune, a phenomenon called *herd immunity,* outbreaks are limited to sporadic cases because there are not enough susceptible individuals to support the spread of epidemics.

The principal vaccines used to prevent bacterial and viral diseases in the United States are listed in **Table 18.1** and **Table 18.2**. Recommendations for childhood immunizations against some of these diseases are given in **Table 18.3**. American travelers who might be exposed to cholera, yellow fever, or other diseases not endemic in the United States can obtain current immunization recommendations from the U.S. Public Health Service and local public health agencies.

CHECK YOUR UNDERSTANDING

✓ Vaccination is often the only feasible way to control most viral diseases; why is this? **18-2**

Types of Vaccines and Their Characteristics

There are now several basic types of vaccine. Some of the newer vaccines take full advantage of knowledge and technology developed in recent years.

Attenuated whole-agent vaccines use living but attenuated (weakened) microbes. Live vaccines more closely mimic an actual infection. Lifelong immunity, especially with viruses, is often achieved without booster immunizations, and an effectiveness

Table 18.1 Principal Vaccines Used in the United States to Prevent Bacterial Diseases in Humans

Disease	Vaccine	Recommendation	Booster
Diphtheria	Purified diphtheria toxoid	See Table 18.3.	Every 10 years for adults
Meningococcal meningitis	Purified polysaccharide from *Neisseria meningitidis*	For people with substantial risk of infection. Recommended for college freshmen, especially if living in dormitories.	Need not established
Pertussis (whooping cough)	Killed whole or acellular fragments of *Bordetella pertussis*	Children prior to school age; see Table 18.3.	For high-risk adults; available for ages 10–18 years
Pneumococcal pneumonia	Purified polysaccharide from 7 strains of *Streptococcus pneumoniae*	For adults with certain chronic diseases; people over 65; children 2–23 months.	None if first dose administered ≥ 24 months
Tetanus	Purified tetanus toxoid	See Table 18.3.	Every 10 years for adults
Haemophilus influenzae type b meningitis	Polysaccharide from *Haemophilus influenzae* type b conjugated with protein to enhance effectiveness	Children prior to school age; see Table 18.3.	None recommended

rate of 95% is not unusual. This long-term effectiveness probably occurs because the attenuated viruses replicate in the body, increasing the original dose and acting as a series of secondary (booster) immunizations.

Examples of attenuated vaccines are the Sabin polio vaccine and those used against measles, mumps, and rubella (MMR). The widely used vaccine against the tuberculosis bacillus and certain of the newly introduced, orally administered typhoid vaccines contain attenuated bacteria. Attenuated microbes are usually derived from mutations accumulated during long-term artificial culture. A danger of such vaccines is that the live microbes can backmutate to a virulent form (discussed on page 506). Attenuated vaccines are not recommended for people whose immune systems are compromised. If available, inactivated vaccines are substituted.

Inactivated whole-agent vaccines use microbes that have been killed, usually by formalin or phenol. Inactivated virus vaccines used in humans include those against rabies (animals sometimes receive a live vaccine considered too hazardous for humans), influenza (**Figure 18.1**), and polio (the Salk polio vaccine). Inactivated bacterial vaccines include those for pneumococcal pneumonia and cholera. Several long-used inactivated vaccines that are being replaced for most uses by newer, more effective types are those for pertussis (whooping cough) and typhoid.

Toxoids, which are inactivated toxins, are vaccines directed at the toxins produced by a pathogen. The tetanus and diphtheria toxoids have long been part of the standard childhood immunization series. They require a series of injections for full immunity, followed by boosters every 10 years. Many older adults have not received boosters; they are likely to have low levels of protection.

Figure 18.1 Influenza viruses are grown in embryonated eggs. (See Figure 13.7, page 377.) The viruses will be inactivated to make a vaccine.

Q Could this method of viral cultivation be a problem for people who are allergic to eggs?

Subunit vaccines use only those antigenic fragments of a microorganism that best stimulate an immune response. Subunit vaccines that are produced by genetic modification techniques, meaning that other microbes are programmed to produce the desired antigenic fraction, are called **recombinant vaccines.** For example, the vaccine against the hepatitis B virus consists of a portion of the viral protein coat that is produced by a genetically modified yeast.

Table 18.2 Principal Vaccines Used in the United States to Prevent Viral Diseases in Humans

Disease	Vaccine	Recommendation	Booster
Influenza	Injected vaccine, inactivated virus (nasally administered vaccine with attenuated virus is now available for some)	For chronically ill, including children over 6 months. Adults over age 65. Healthy children aged 6–23 months (because higher risk of related hospitalizations). Health care workers and others in contact with high risk groups. Healthy persons aged 5–49 years can receive intranasal vaccine.	Annual
Measles	Attenuated virus	For infants aged 15 months.	Adults if exposed during outbreak
Mumps	Attenuated virus	For infants aged 15 months.	Adults if exposed during outbreak
Rubella	Attenuated virus	For infants aged 15 months; for women of childbearing age who are not pregnant.	Adults if exposed during outbreak
Chickenpox	Attenuated virus	For infants aged 12 months.	(Duration of immunity not known)
Poliomyelitis	Killed virus	For children, see Table 18.3; for adults, as risk to exposure warrants.	(Duration of immunity not known)
Rabies	Killed virus	For field biologists in contact with wildlife in endemic areas; for veterinarians; for people exposed to rabies virus by bites.	Every 2 years
Hepatitis B	Antigenic fragments of virus	For infants and children, see Table 18.3; for adults, especially health care workers, homosexual men, injecting drug users, heterosexual people with multiple partners, and household contacts of hepatitis B carriers.	Duration of protection at least 7 years; need for boosters uncertain
Hepatitis A	Inactivated virus	Mostly for travel to endemic areas and protecting contacts during outbreaks.	Duration of protection estimated at about 10 years
Smallpox	Live vaccinia virus	Certain military and health care personnel.	Duration of protection estimated at about 3 to 5 years
Herpes zoster	Attenuated virus	Adults over age 60.	None recommended
Human papilloma virus	Antigenic fragments of virus	All females under age 26.	Duration at least 5 years

Subunit vaccines are inherently safer because they cannot reproduce in the recipient. They also contain little or no extraneous material and therefore tend to produce fewer adverse effects. Similarly, it is possible to separate the fractions of a disrupted bacterial cell, retaining the desired antigenic fractions. The newer **acellular vaccines** for pertussis use this approach.

Conjugated vaccines have been developed in recent years to deal with the poor immune response of children to vaccines based on capsular polysaccharides. As shown in Figure 17.6 (page 484), polysaccharides are T-independent antigens; children's immune systems do not respond well to these antigens until the age of 15 to 24 months. Therefore, the polysaccharides are combined with proteins such as diphtheria toxoid; this approach has led to the very successful vaccine for *Haemophilus influenzae* type b, which gives significant protection even at 2 months.

Nucleic acid vaccines, often called *DNA vaccines*, are among the newest and most promising vaccines. Experiments with animals show that plasmids of "naked" DNA injected into muscle results in the production of the protein antigen encoded in the DNA. The injection can be made by conventional needle or, more efficiently, by the "gene gun" method described in Chapter 9, page 253, and Figure 9.6, which delivers the vaccine into many skin cell nuclei. The protein antigens are carried to the red bone marrow and stimulate both humoral and cellular immunity. They also tend to be expressed for extended times, with good immunological memory. However, vaccines based on polysaccharide capsules of bacteria cannot be made by this method.

Two DNA vaccines for animals have been approved; one that protects horses from West Nile virus, and another that protects domestically reared salmon from a serious viral disease. Clinical tests in humans are underway testing DNA vaccines for a number of different diseases; human immunization with some of these vaccines can be expected in the next few years. Such vaccines would have particular advantages for the less developed parts of the world. The "gene gun" would eliminate the need for large supplies of syringes and needles, and these vaccines would not require refrigeration. Manufacturing operations for such vaccines are very similar for different

Table 18.3 Recommended Immunization Schedule for Persons Aged 0–6 Years—United States, 2008 (CDC)

Age ▶ / Vaccine ▼	Birth	1 month	2 months	4 months	6 months	12 months	15 months	18 months	19–23 months	2–3 years	4–6 years
Hepatitis B	HepB	HepB			HepB						
Rotavirus			Rota	Rota	Rota						
Diphtheria, Tetanus, Pertussis			DTaP	DTaP	DTaP		DTaP				DTaP
***Haemophilus influenzae* type b**			Hib	Hib	Hib	Hib					
Pneumococcal*			PCV	PCV	PCV	PCV				PPV	
Inactivated Poliovirus			IPV	IPV	IPV						IPV
Influenza					Influenza (Yearly)						
Measles, Mumps, Rubella						MMR					MMR
Varicella						Varicella					Varicella
Hepatitis A†						HepA (2 doses)					
Meningococcal‡										MCV4	

Note: Vaccines are listed under routinely recommended ages. Bars indicate range of recommended ages for immunization. For those who fall behind or start late, see the catch-up schedule. Additional information at www.cdc.gov/vaccines/rec/schedules/

* PCV = Pneumococcal conjugate vaccine, PPV = Pneumococcal polysaccharide vaccine.

† The two doses at least 6 mo. apart.

‡ Meningococcal conjugate vaccine (MCV4) for children aged 2–10 years with defective immune systems and certain other high risk situations.

diseases, which should lower costs. **Animation** Vaccines: Types. **www.microbiologyplace.com**

CHECK YOUR UNDERSTANDING

- Experience has shown that attenuated vaccines tend to be more effective than inactivated vaccines. Why? **18-3**
- Which is more likely to be useful in preventing a disease caused by an encapsulated bacterium such as the pneumococcus: a subunit vaccine or a nucleic acid vaccine? **18-4**

The Development of New Vaccines

An effective vaccine is the most desirable method of disease control. It prevents the targeted disease from ever occurring at all in an individual, and it is generally the most economical. This is especially important in developing parts of the world.

Although interest in vaccine development declined with the introduction of antibiotics, it has intensified in recent years. Fear of litigation contributed to the decrease in the development of new vaccines in the United States. However, passage of the National Childhood Vaccine Injury Act in 1986, which limits the liability of vaccine manufacturers, has now helped reverse this trend. Even so, pharmaceutical companies find that the most profitable drugs are those that must be taken daily for extended periods, for example, drugs taken for high blood pressure. In contrast, a vaccine that is required for a few injections or even only once in a lifetime is inherently less attractive.

Historically, vaccines could be developed only by growing the pathogen in usefully large amounts. The early successful viral vaccines were developed by animal cultivation. The vaccinia virus for smallpox was grown on the shaved bellies of calves, for example.

The introduction of vaccines against polio, measles, mumps, and a number of other viral diseases that would not grow in anything but a living human awaited the development of cell culture techniques. Cell cultures from human sources or, more often, from animals such as monkeys that are closely related to humans enabled growth of these viruses on a large scale. A convenient animal that will grow many viruses is the chick embryo (see Figure 13.7, page 377). Viruses for several vaccines (influenza, for example) are grown this way (see Figure 18.1). Interestingly, the first vaccine against hepatitis B virus used viral antigens extracted from the blood of chronically infected humans because no other source was available.

A World Health Problem

As you read through this box, you will encounter a series of questions that public health scientists ask themselves as they try to reduce the occurrence of diseases. Try to answer each question before going to the next one.

1. On May 14, a 17-year-old girl developed a fever and tiny red spots with blue-white centers inside her mouth (**Figure A**). She developed a rash on her face on May 16; the rash spread over her trunk and extremities. Subsequently, a 2-year-old developed a fever and pneumonia. In all, 34 people from her church developed a maculopapular rash and fever (≥38°C), and at least one of the following: fever, cough, conjunctivitis, coldlike symptoms.

   What is the disease? *(Hint:* See Table 21.1 on page 589.)

2. Measles was confirmed by testing for IgM measles antibodies. Measles is a highly contagious viral illness that can cause pneumonia, diarrhea, encephalitis, and death.

   What do you need to know?

3. The index patient had traveled to Romania for 2 weeks. She and the other infected people had not been vaccinated against measles.

   Why didn't more people get measles?

4. In 1920, prior to development of the measles vaccine, nearly 500,000 cases of measles occurred, with over 7500 deaths, in the United States. In 2007, only 30 cases of measles were reported in the United States (**Figure B**).

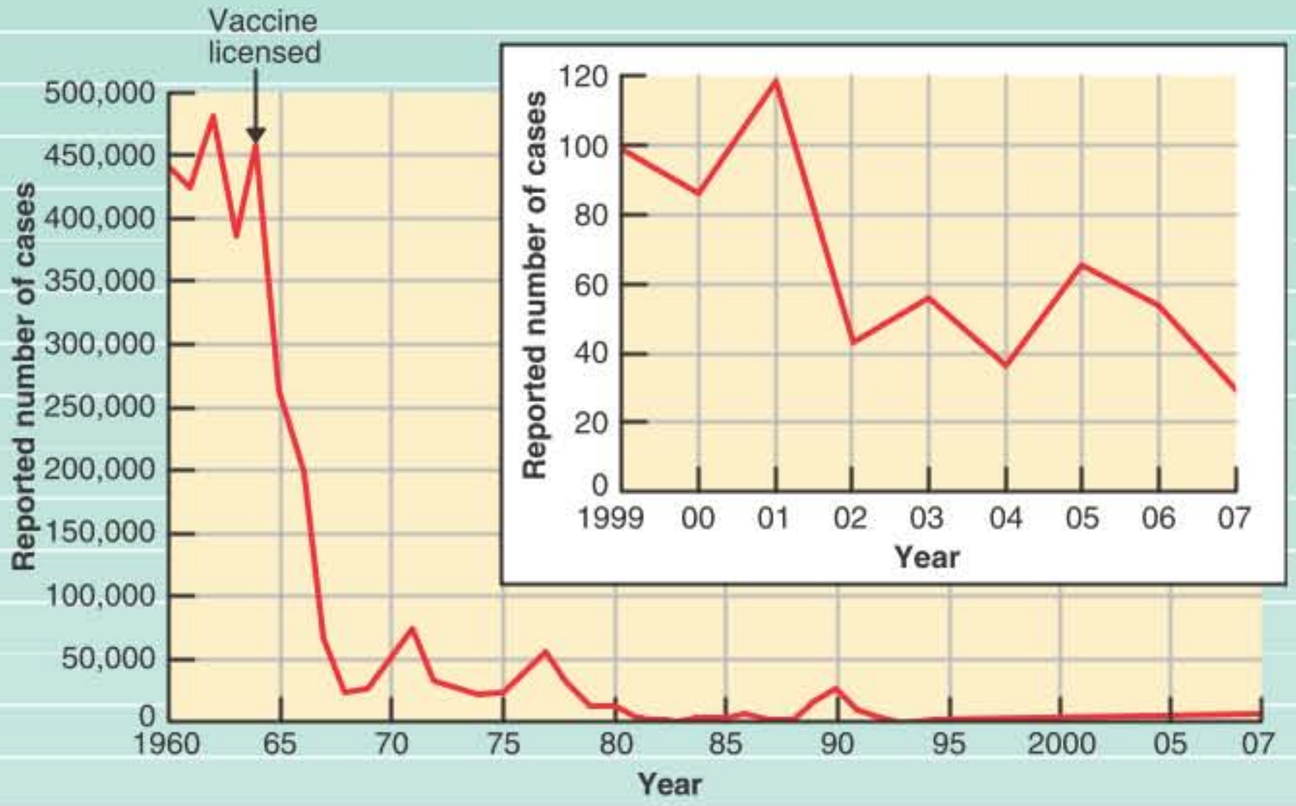


Figure B Reported numbers of measles cases in the United States, 1960–2007. (CDC, 2008)

However, measles is still endemic in many countries (**Figure C**). Worldwide, there are 70 million cases each year. Measles is still one of the top 20 causes of death, killing 600 children per day.

What would happen if we stopped vaccinating against measles?

5. If there were no vaccines, there would be many more cases of disease. Along with more disease, there would be serious sequelae and more deaths. Some vaccine-preventable diseases are still quite prevalent in other parts of the world. As occurred in this case, travelers can unknowingly bring these diseases into the United States, and if we were not protected by vaccinations, these diseases could quickly spread throughout the population, causing epidemics here.

The Measles Initiative is a partnership—led by the American Red Cross, the United Nations Foundation, UNICEF, the U.S. Centers for Disease Control and Prevention, and the World Health Organization—committed to reducing measles deaths worldwide. The Measles Initiative has supported the vaccination of more than 400 million children in over 50 countries. In 2000, measles caused approximately 757,000 deaths, mostly in children under 5. By 2006, measles deaths were reduced to 242,000 people worldwide.

Source: Adapted from *MMWR* 54(42):1073–1075, October 28, 2005.

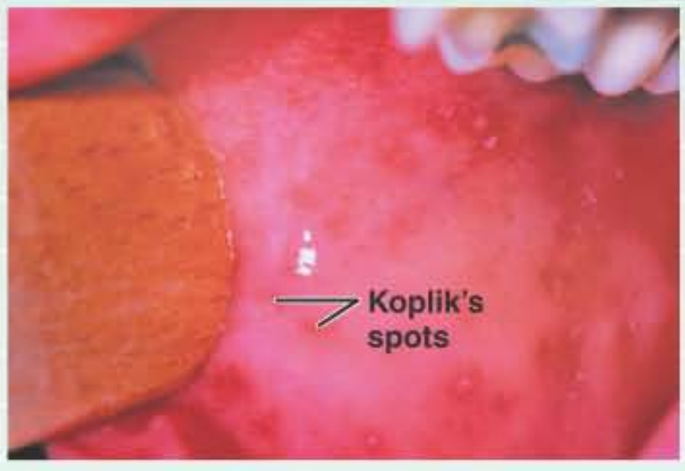


Figure A Koplik's spots inside the cheeks.

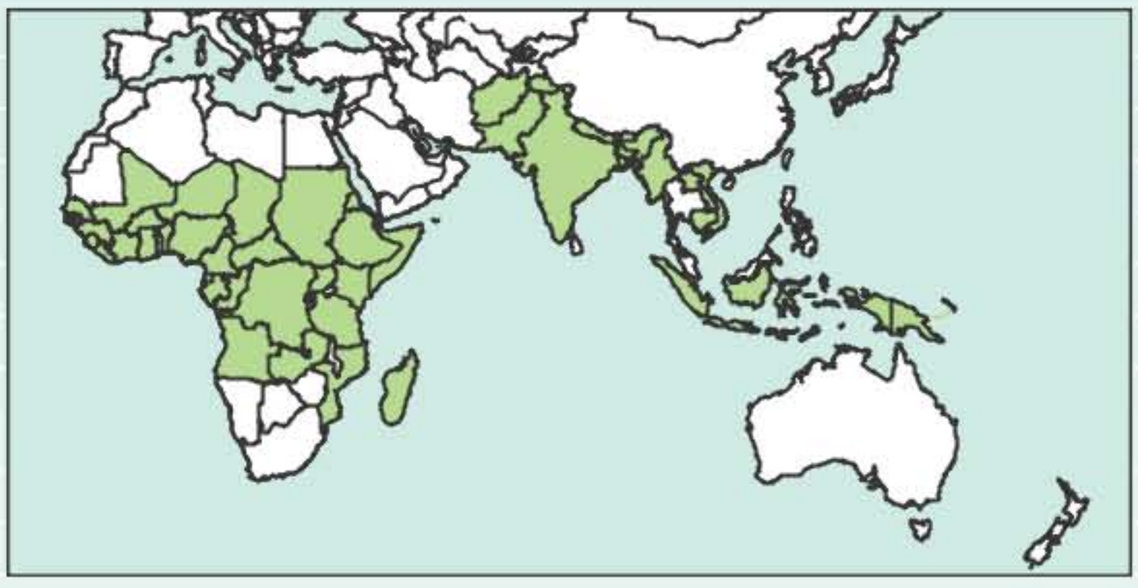


Figure C Countries with the highest measles mortality.

Recombinant vaccines and DNA vaccines do not need a cell or animal host to grow the vaccine's microbe. This avoids a major problem with certain viruses that so far have not been grown in cell culture—hepatitis B, for example.

Plants are also a potential source for vaccines. There have already been human trials with potatoes that have been engineered to produce antigenic proteins from certain pathogenic bacteria and viruses. It is more likely, however, that plants for this purpose will not be used directly as food, but as a production system for doses of antigenic proteins that would be taken orally as pills. Oral vaccines would be welcomed for many reasons even beyond eliminating a need for injections. For one, they would be especially effective in protecting against the diseases caused by pathogens invading the body through the mucous membranes. This obviously includes intestinal diseases such as cholera, but the pathogens causing AIDS, influenza, and some other nonintestinal diseases initially invade the body through mucous membranes elsewhere, such as the nose, genitals, and lungs. Tobacco plants are a leading candidate for this use because they are unlikely to contaminate the food chain.

The so-called golden age of immunology occurred from about 1870 to 1910, when most of the basic elements of immunology were discovered and several important vaccines were developed. We may soon be entering another golden age, in which new technologies are brought to bear on emerging infectious diseases and problems arising from the decreasing effectiveness of antibiotics. It is remarkable that there are no useful vaccines against chlamydias, fungi, protozoa, or helminthic parasites of humans. Moreover, vaccines for some diseases, such as cholera and tuberculosis, are not reliably protective. At present, vaccines for at least 75 diseases are under development, ranging from those for prominent deadly diseases such as AIDS and malaria to such commonplace conditions as earaches. But we will probably find that the easy vaccines have already been made.

Infectious diseases are not the only possible target of vaccines. Researchers are investigating vaccines' potential for treating and preventing cocaine addiction, Alzheimer disease, and cancer and for contraception.

Work is underway to improve effectiveness of antigens. For example, chemicals added for this purpose, called **adjuvants** (from the Latin *adjuvare*, meaning to help), greatly improve the effectiveness of many antigens. Alum is the adjuvant with the longest history of use. It causes local inflammatory reactions that apparently increase vaccine effectiveness. Other adjuvants have recently been registered for use, including an oil-based substance, MF59, and virosomes. These are not inflammatory in their activity but mimic certain bacterial components and facilitate transport to lymph nodes and uptake by antigen-presenting cells.

Currently, nearly 20 separate injections are recommended for infants and children, sometimes requiring three or more at one appointment. Developing additional multiple combinations of vaccines would be of some help. The U.S. Food and Drug Administration (FDA) has recently approved such a combination for five childhood diseases. Delivery in ways other than by needle would also be a desirable advance. Many have already received injection by high-pressure "guns," which are commonly used for mass inoculations, and an intranasal spray for influenza is now available. Most current vaccines have primarily induced humoral, or antibody-based, immunity. Vaccines effective against diseases such as HIV infection, tuberculosis, and malaria will require induction of effective cellular immunity as well. These requirements are not necessarily mutually exclusive.

CHECK YOUR UNDERSTANDING

- ✓ Which type of vaccine did Louis Pasteur develop, whole-agent, recombinant, or DNA? **18-5**
- ✓ What is the derivation of the word *adjuvant*? **18-6**

Safety of Vaccines

We have seen how variolation, the first attempt to provide immunity to smallpox, sometimes caused the disease it was intended to prevent. At the time, however, the risk was considered very worthwhile. As you will see later in this book, the oral polio vaccine on rare occasions may *cause* the disease. In 1999, a vaccine to prevent infant diarrhea caused by rotaviruses was withdrawn from the market because several recipients developed a life-threatening intestinal obstruction. However, public reaction to such risks has changed; most parents have never seen a case of polio or measles and therefore tend to view the risk of these diseases as a remote abstraction. Moreover, reports or rumors of harmful effects often lead people to avoid certain vaccines for themselves or their children. In particular, a possible connection between the MMR vaccine and autism has received widespread publicity. Autism is a poorly understood developmental condition that causes a child to withdraw from reality. Because autism is usually diagnosed at the age of 18 to 30 months, about the time vaccine immunization schedules are nearing completion, some people have attempted to make a cause-and-effect connection. Medically, however, most experts agree that autism is a condition with a major genetic component and begins before birth. Extensive scientific surveys have provided no evidence to support a connection between the usual childhood vaccines and autism or any other disease condition. Some experts even recommend again introducing the rotavirus vaccine that was withdrawn in the United States, maintaining that it would be well justified on a risk-versus-benefit calculation in much of the underdeveloped world. No vaccine will ever be perfectly safe or perfectly effective—neither is any antibiotic or most other drugs, for that matter. Nevertheless, vaccines still remain the safest and most effective means of preventing infectious disease in children.

CHECK YOUR UNDERSTANDING

- ✓ What is the name of a currently used oral vaccine that occasionally causes the disease it is intended to prevent? **18-7**

Diagnostic Immunology

LEARNING OBJECTIVES

18-8 Differentiate sensitivity from specificity in a diagnostic test.

18-9 Define *monoclonal antibodies*, and identify their advantage over conventional antibody production.

18-10 Explain how precipitation reactions and immunodiffusion tests work.

18-11 Differentiate direct from indirect agglutination tests.

18-12 Differentiate agglutination from precipitation tests.

18-13 Define *hemagglutination*.

18-14 Explain how a neutralization test works.

18-15 Differentiate precipitation from neutralization tests.

18-16 Explain the basis for the complement-fixation test.

18-17 Compare and contrast direct and indirect fluorescent-antibody tests.

18-18 Explain how direct and indirect ELISA tests work.

18-19 Explain how Western blotting works.

18-20 Explain the importance of monoclonal antibodies.

Throughout most of history, diagnosing a disease was essentially a matter of observing a patient's signs and symptoms. The writings of ancient and medieval physicians left descriptions of many diseases that are recognizable even today. Essential elements of diagnostic tests are sensitivity and specificity. **Sensitivity** is the probability that the test is reactive if the specimen is a true positive. **Specificity** is the probability that a positive test will *not* be reactive if a specimen is a true negative.

Immunologic-Based Diagnostic Tests

Knowledge of the high specificity of the immune system soon suggested that this might be used in diagnosing diseases. In fact, it was an accidental observation that led to one of the first diagnostic tests for an infectious disease. More than 100 years ago, Robert Koch was trying to develop a vaccine against tuberculosis. He observed that when guinea pigs with the disease were injected with a suspension of *Mycobacterium tuberculosis*, the site of the injection became red and slightly swollen a day or two later. You may recognize this symptom as a positive result for the widely used tuberculin skin test (see Figure 24.10, page 684)—many colleges and universities require the test as part of admission procedures. Koch, of course, had no idea of the mechanism of cell-mediated immunity that caused this phenomenon, nor did he know of the existence of antibodies.

Since the time of Robert Koch, immunology has given us many other invaluable diagnostic tools, most of which are based on interactions of humoral antibodies with antigens. A known antibody can be used to identify an *unknown* pathogen (antigen) by its reaction with it. This reaction can be reversed, and a *known* pathogen can be used, for example, to determine the presence of an unknown antibody in a person's blood—which would determine whether he or she had immunity to the pathogen. One problem that must be overcome in antibody-based diagnostic tests is that antibodies cannot be seen directly. Even at magnifications of well over 100,000×, they appear only as fuzzy, ill-defined particles (see Figure 17.3c on page 480). Therefore, their presence must be established indirectly. We will describe a number of ingenious solutions to this problem.

Other problems that had to be overcome were that antibodies produced in an animal were mixed with numerous other antibodies produced in that animal and the quantities of any particular antibody were severely limited.

CHECK YOUR UNDERSTANDING

✓ What property of the immune system suggested its use as an aid for diagnosing disease: specificity or sensitivity? **18-8**

Monoclonal Antibodies

As soon as it was determined that antibodies were produced by specialized cells (B cells), it was understood that these were a potential source of a single type of antibody. If such a B cell producing a single type of antibody could be isolated and cultivated, it would be able to produce the desired antibody in nearly unlimited quantities and without contamination by other antibodies. Unfortunately, a B cell reproduces only a few times under the usual cell culture conditions. This problem was largely solved with the discovery of a method to isolate and indefinitely cultivate B cells capable of producing a single type of antibody. Neils Jerne, Georges Köehler, and César Milstein made this discovery in 1975, for which they were awarded a Nobel prize.

Scientists have long observed that antibody-producing B cells may become cancerous. In this case, their proliferation is unchecked, and they are called *myelomas*. These cancerous B cells can be isolated and propagated indefinitely in cell culture. Cancer cells, in this sense, are "immortal." The breakthrough came in combining an "immortal" cancerous B cell with an antibody-producing normal B cell. When fused, this combination is termed a **hybridoma.**

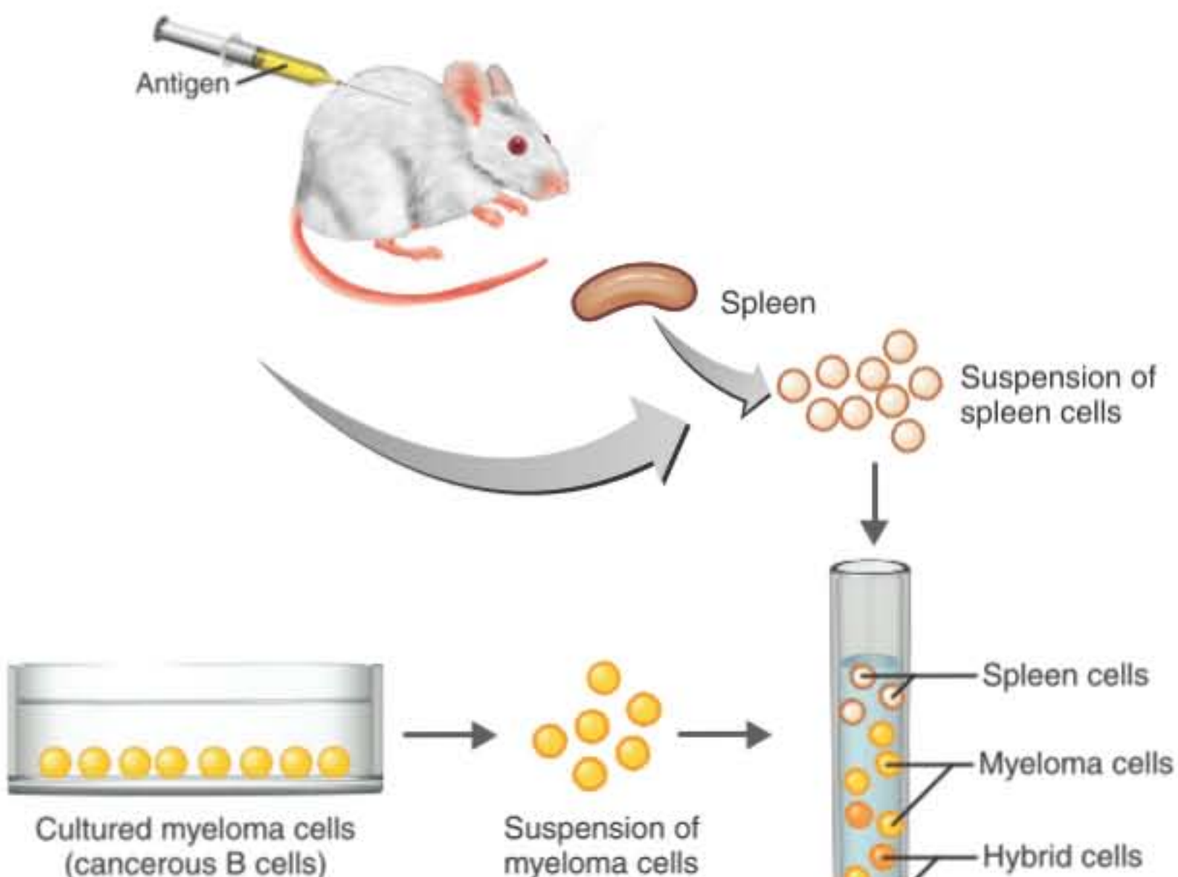
When a hybridoma is grown in culture, its genetically identical cells continue to produce the type of antibody characteristic of the ancestral B cell. The importance of the technique is that clones of the antibody-secreting cells now can be maintained indefinitely in cell culture and can produce immense quantities of identical antibody molecules. Because all of these antibody molecules are produced by a single hybridoma clone, they are called **monoclonal antibodies,** or **Mabs** (Figure 18.2).

Monoclonal antibodies are useful for three reasons: they are uniform; they are highly specific; and they can be produced readily in large quantities. Because of these qualities, Mabs have assumed enormous importance as diagnostic tools. For instance, commercial kits use Mabs to recognize several bacterial pathogens, and nonprescription pregnancy tests use Mabs to indicate the presence of a hormone excreted only in the urine of a pregnant woman (see Figure 18.13, page 517).

Figure 18.2

FOUNDATION FIGURE The Production of Monoclonal Antibodies

An appreciation of how monoclonal antibodies, an important advance in medicine, are produced is helpful for understanding the functions and applications of many common diagnostic and therapeutic tools that will be discussed in this and subsequent chapters.



1. A mouse is injected with a specific antigen that will induce antibodies against that antigen.
2. The spleen of the mouse is removed and homogenized into a cell suspension. The suspension includes B cells that produce antibodies against the injected antigen.
3. The spleen cells are then mixed with myeloma cells that are capable of continuous growth in culture but have lost the ability to produce antibodies. Some of the antibody-producing spleen cells and myeloma cells fuse to form hybrid cells. These hybrid cells are now capable of growing continuously in culture while producing antibodies.
4. The mixture of cells is placed in a selective medium that allows only hybrid cells to grow.
5. Hybrid cells proliferate into clones called hybridomas. The hybridomas are screened for production of the desired antibody.
6. The selected hybridomas are then cultured to produce large quantities of monoclonal antibodies.

Key Concept

The fusion of cultured myeloma cells (cancerous B cells) with antibody-producing spleen cells forms a hybridoma. Hybridomas can be cultured to produce large quantities of identical antibodies, called monoclonal antibodies.

Monoclonal antibodies are also being used therapeutically to overcome unwanted effects of the immune system. For example, *muromonab-CD3* has been used since 1986 to minimize rejection of kidney transplants. For these purposes, monoclonal antibodies are prepared that react with the T cells that are responsible for rejecting the transplanted tissue. The Mabs suppress the T cell activity.

The use of Mabs is revolutionizing the treatment of many illnesses. The FDA has approved several for the treatment of

specific diseases. The inflammation of rheumatoid arthritis and the intestinal inflammatory condition Crohn's disease can be treated with *infliximab (Remicade)* or *entanercept (Enbrel)*, which target and block the action of a cause of the inflammation, tumor necrosis factor alpha (see page 492). A cancer of the lymphatic system, non-Hodgkin's lymphoma, can be treated with a combination of Mabs, *ibritumonab (Zevalin)* and *rituximab (Rituxan)*. These target and destroy cancer cells but are used only when other treatments have proved unsuccessful. Breast cancer can be treated with some success with *trastuzumab (Herceptin)*. This Mab binds to a specific site called the HER-2 receptor, which occurs in about 30% of women; this limits the spread of the cancer.

The therapeutic use of Mabs had been limited because these antibodies once were produced only by mouse (murine) cells. The immune systems of patients reacted against the foreign mouse proteins, leading to rashes, swelling, and even occasional kidney failure, plus the destruction of the Mabs. For example, the success of *muromonab-CD3* in minimizing tissue rejection was severely limited by side effects related to the administration of the foreign (murine) fraction of the monoclonal antibody.

In recognition of this problem, researchers are developing new generations of Mabs that are less likely to cause side effects due to their "foreignness." Essentially, the more human the antibody, the more successful it is likely to be. Researchers are exploring several approaches.

Chimeric monoclonal antibodies use genetically modified mice to make a human–murine hybrid. The variable part of the antibody molecule, including the antigen-binding sites (see Figure 17.3a), is murine. The remainder of the antibody molecule, the constant region, has been derived from a human source. These Mabs are about 66% human. An example is rituximab.

Humanized antibodies are constructed so that the murine portion is limited to the antigen-binding sites. The balance of the variable region and all of the constant region are derived from human sources. Such Mabs are about 90% human. Examples are alemtuzumab and trastuzumab.

The eventual goal is to develop **fully human antibodies.** One approach is to genetically modify mice to contain human antibody genes. The mice would produce antibodies that are fully human; in some cases, it might even be possible to produce an antibody that is an exact match to the patient.

It is also possible that Mab therapies may succeed so well that it would be difficult to produce them in sufficient volumes. Several potential solutions to this problem are under investigation. For example, the use of mice could be avoided entirely by using bacteriophages to insert desired genes into bacteria, which would be able to produce the desired Mabs on an industrial scale. Another approach to the problem is to genetically modify animals that can secrete the Mabs in their milk. Genetic alteration of plants to produce Mabs is another possible avenue to large-scale production.

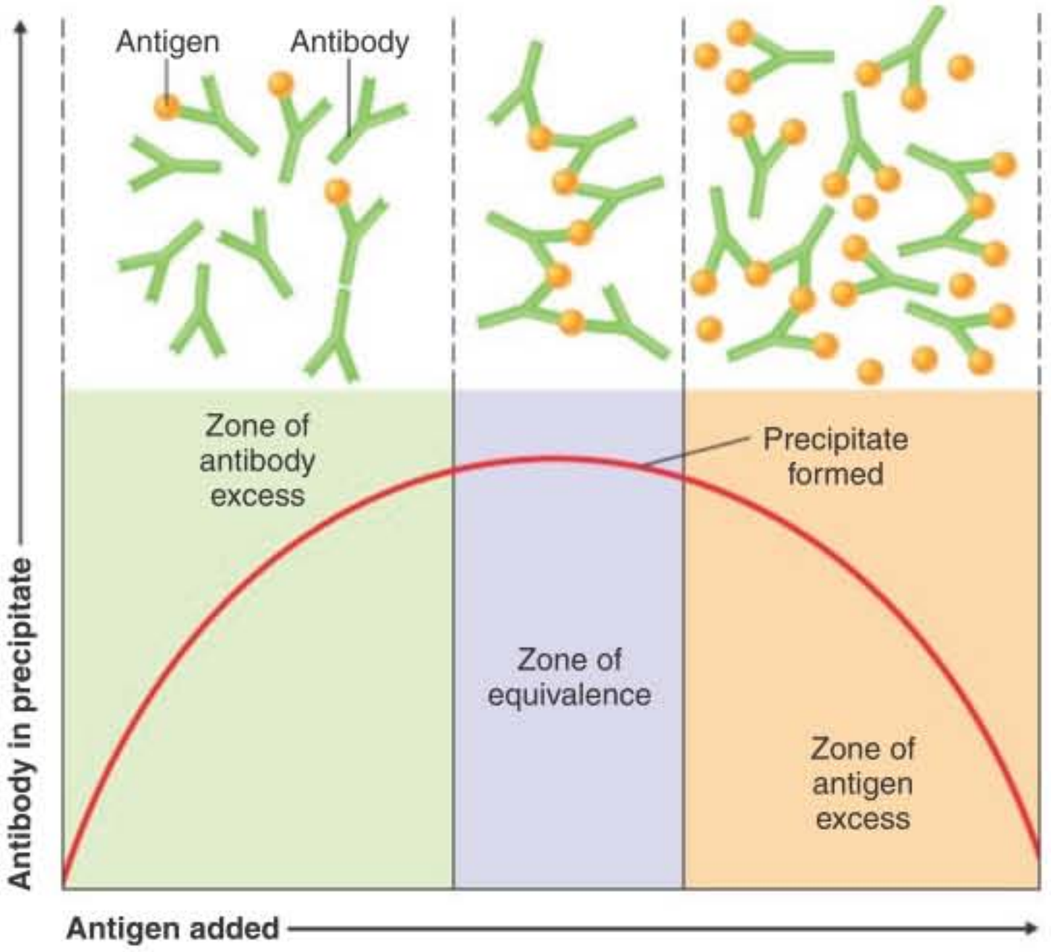


Figure 18.3 A precipitation curve. The curve is based on the ratio of antigen to antibody. The maximum amount of precipitate forms in the zone of equivalence, where the ratio is roughly equivalent.

Q How does precipitation differ from agglutination?

CHECK YOUR UNDERSTANDING

✓ The blood of an infected cow would have a considerable amount of antibodies against the infectious pathogen in its blood. How would an equivalent amount of monoclonal antibodies be more useful? 18-9

Precipitation Reactions

Precipitation reactions involve the reaction of soluble antigens with IgG or IgM antibodies to form large, interlocking molecular aggregates called *lattices.*

Precipitation reactions occur in two distinct stages. First, the antigens and antibodies rapidly form small antigen–antibody complexes. This interaction occurs within seconds and is followed by a slower reaction, which may take minutes to hours, in which the antigen–antibody complexes form lattices that precipitate from solution. Precipitation reactions normally occur only when the ratio of antigen to antibody is optimal. Figure 18.3 shows that no visible precipitate forms when either component is in excess. The optimal ratio is produced when separate solutions of antigen and antibody are placed adjacent to each other and allowed to diffuse together. In a **precipitin ring test** (Figure 18.4), a cloudy line of precipitation (ring) appears in the area in which the optimal ratio has been reached (the *zone of equivalence*).

Immunodiffusion tests are precipitation reactions carried out in an agar gel medium, on either a Petri plate or a

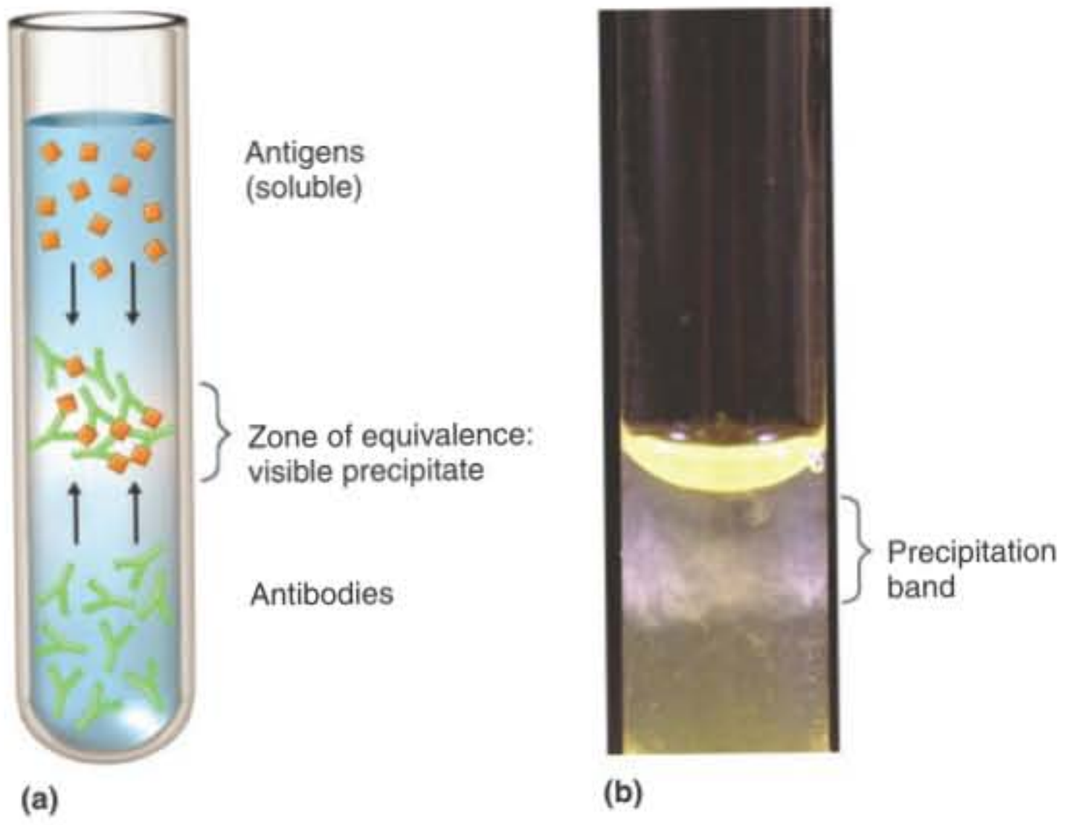


Figure 18.4 The precipitin ring test. (**a**) This drawing shows the diffusion of antigens and antibodies toward each other in a small-diameter test tube. Where they reach equal proportions, in the zone of equivalence, a visible line or ring of precipitate is formed. (**b**) A photograph of a precipitin band.

Q What causes the visible line?

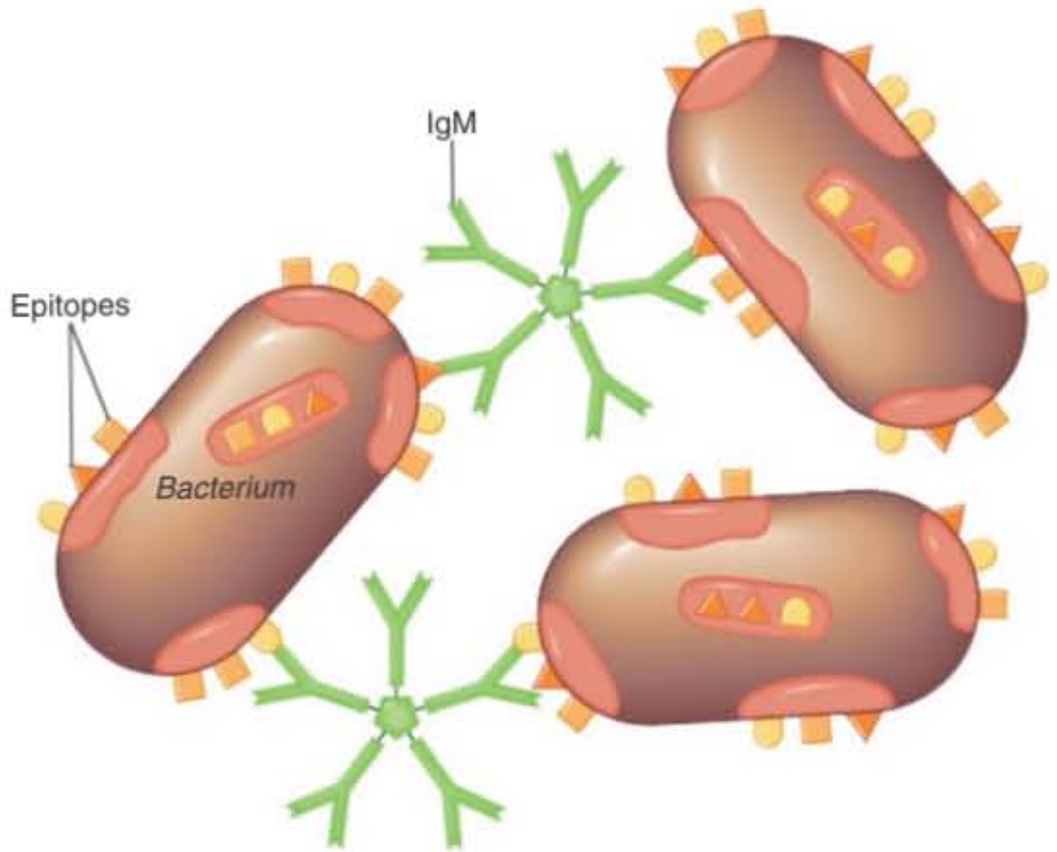


Figure 18.5 An agglutination reaction. When antibodies react with epitopes on antigens carried on neighboring cells, such as these bacteria (or red blood cells), the particulate antigens (cells) agglutinate. IgM, the most efficient immunoglobulin for agglutination, is shown here, but IgG also participates in agglutination reactions.

Q Draw an agglutination reaction involving IgG.

microscope slide. A line of visible precipitate develops between the wells at the point where the optimal antigen–antibody ratio is reached.

Other tests use electrophoresis to speed up the movement of antigen and antibody in a gel, sometimes in less than an hour, with this method. The techniques of immunodiffusion and electrophoresis can be combined in a procedure called **immunoelectrophoresis.** The procedure is used in research to separate proteins in human serum and is the basis of certain diagnostic tests. It is an essential part of the Western blot test used in AIDS testing (see Figure 10.12, page 289 and page 516).

CHECK YOUR UNDERSTANDING

✓ Why does the reaction of a precipitation test become visible only in a narrow range? 18-10

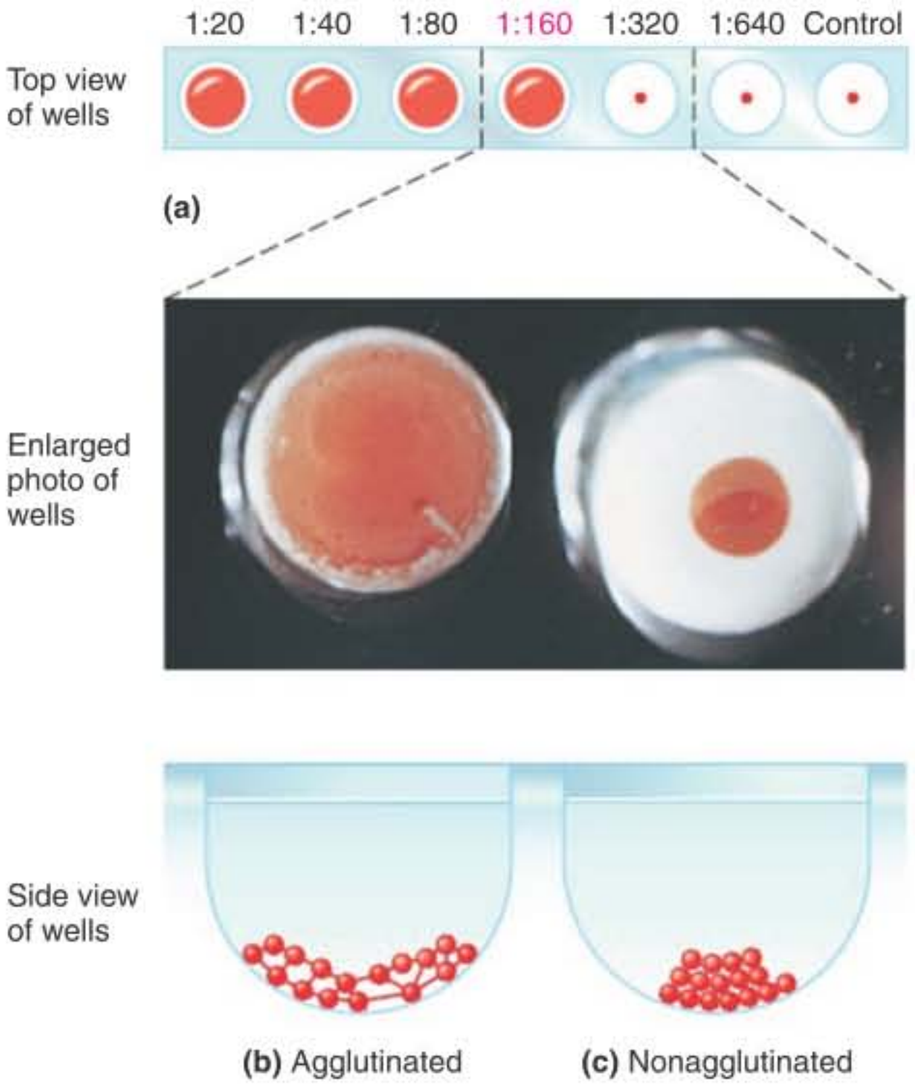
Agglutination Reactions

Whereas precipitation reactions involve *soluble* antigens, agglutination reactions involve either *particulate* antigens (particles such as cells that carry antigenic molecules) or soluble antigens adhering to particles. These antigens can be linked together by antibodies to form visible aggregates, a reaction called **agglutination** (Figure 18.5). Agglutination reactions are very sensitive, relatively easy to read (see Figure 10.10, page 287), and available in great variety. Agglutination tests are classified as either direct or indirect.

Direct Agglutination Tests

Direct agglutination tests detect antibodies against relatively large cellular antigens, such as those on red blood cells, bacteria, and fungi. At one time they were carried out in a series of test tubes, but now they are usually done in plastic *microtiter plates,* which have many shallow wells that take the place of the individual test tubes. The amount of particulate antigen in each well is the same, but the amount of serum that contains antibodies is diluted, so that each successive well has half the antibodies of the previous well. These tests are used, for example, to test for brucellosis and to separate *Salmonella* isolates into serovars, types defined by serological means.

Clearly, the more antibody we start with, the more dilutions it will take to lower the amount to the point where there is not enough antibody for the antigen to react with. This is the measure of **titer,** or concentration of serum antibody (Figure 18.6). For infectious diseases in general, the higher the serum antibody titer, the greater the immunity to the disease. However, the titer alone is of limited use in diagnosing an existing illness. There is no way to know whether the measured antibodies were generated in response to the immediate infection or to an earlier illness. For diagnostic purposes, *a rise in titer* is significant; that is, the titer is higher later in the course of the disease than at its outset. Also, if it can be demonstrated that the person's blood had no antibody titer before the illness but has a significant titer while the disease is progressing, this change, called **seroconversion,** is also diagnostic. This situation is frequently encountered with HIV infections.



(a) Each well in this microtiter plate contains, from left to right, only half the concentration of serum that is contained in the preceding well. Each well contains the same concentration of particulate antigens, in this instance red blood cells.

(b) In a positive (agglutinated) reaction, sufficient antibodies are present in the serum to link the antigens together, forming a mat of antigen–antibody complexes on the bottom of the well.

(c) In a negative (nonagglutinated) reaction, not enough antibodies are present to cause the linking of antigens. The particulate antigens roll down the sloping sides of the well, forming a pellet at the bottom. In this example, the antibody titer is 160 because the well with a 1:160 concentration is the most dilute concentration that produces a positive reaction.

Figure 18.6 Measuring antibody titer with the direct agglutination test.

Q **What is meant by the term *antibody titer*?**

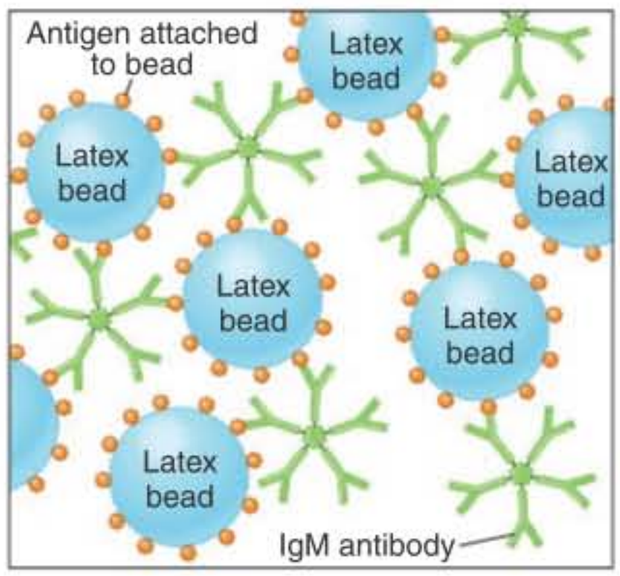
Some diagnostic tests specifically identify IgM antibodies. As discussed in Chapter 17, short-lived IgM is more likely to reflect a response to a current disease condition.

Indirect (Passive) Agglutination Tests

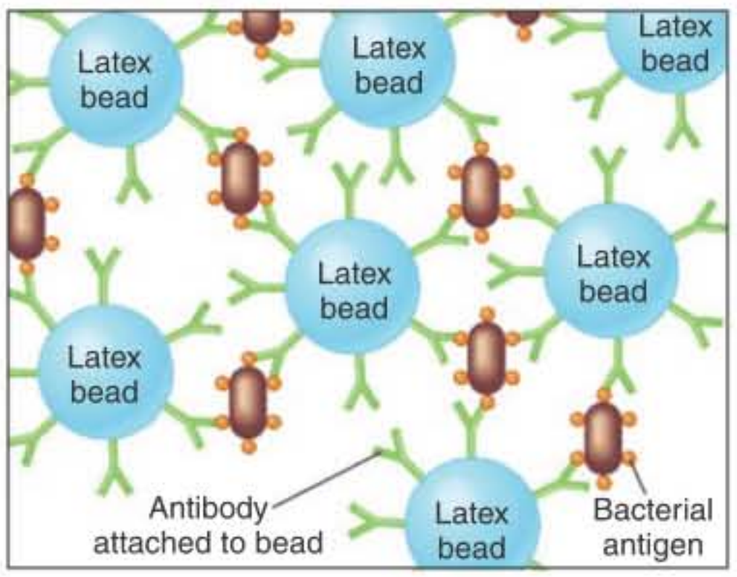
Antibodies against soluble antigens can be detected by agglutination tests if the antigens are adsorbed onto particles such as bentonite clay or, most often, minute latex spheres, each about one-tenth of the diameter of a bacterium. Such tests, known as *latex agglutination tests*, are commonly used for the rapid detection of serum antibodies against many bacterial and viral diseases. In such **indirect (passive) agglutination tests,** the antibody reacts with the soluble antigen adhering to the particles (Figure 18.7). The particles then agglutinate with one another, much as particles do in the direct agglutination tests. The same principle can be applied in reverse by using particles coated with antibodies to

Figure 18.7 Reactions in indirect agglutination tests. These tests are performed using antigens or antibodies coated onto particles such as minute latex spheres.

Q **Differentiate direct from indirect agglutination tests.**



(a) Reaction in a positive indirect test for antibodies. When particles (latex beads here) are coated with antigens, agglutination indicates the presence of antibodies, such as the IgM shown here.



(b) Reaction in a positive indirect test for antigens. When particles are coated with monoclonal antibodies, agglutination indicates the presence of antigens.

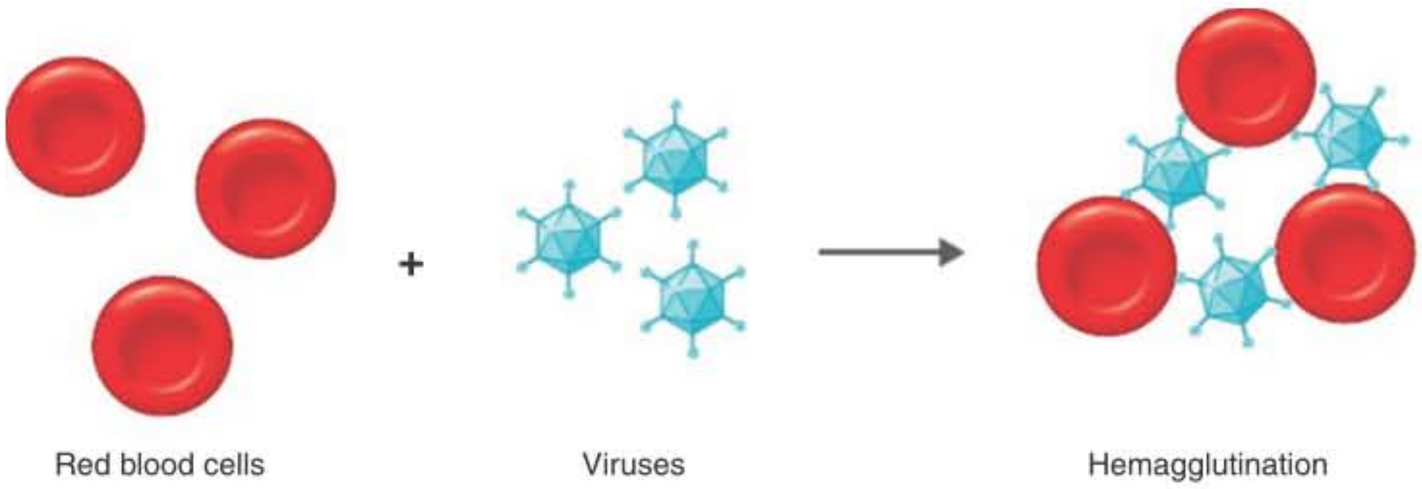


Figure 18.8 Viral hemagglutination. Viral hemagglutination is not an antigen–antibody reaction.

Q What causes agglutination in viral hemagglutination?

detect the antigens against which they are specific. This approach is especially common in tests for the streptococci that cause sore throats. A diagnosis can be completed in about 10 minutes.

Hemagglutination

When agglutination reactions involve the clumping of red blood cells, the reaction is called **hemagglutination.** These reactions, which involve red blood cell surface antigens and their complementary antibodies, are used routinely in blood typing (see Table 19.2, page 527) and in the diagnosis of infectious mononucleosis.

Certain viruses, such as those causing mumps, measles, and influenza, have the ability to agglutinate red blood cells without an antigen–antibody reaction; this process is called **viral hemagglutination** (Figure 18.8). This type of hemagglutination can be inhibited by antibodies that neutralize the agglutinating virus. Diagnostic tests based on such neutralization reactions are discussed in the next section.

CHECK YOUR UNDERSTANDING

- Why wouldn't a direct agglutination test work very well with viruses? 18-11
- Which test detects soluble antigens, agglutination or precipitation? 18-12
- Certain diagnostic tests require red blood cells that clump visibly. What are these tests called? 18-13

Neutralization Reactions

Neutralization is an antigen–antibody reaction in which the harmful effects of a bacterial exotoxin or a virus are blocked by specific antibodies. These reactions were first described in 1890, when investigators observed that immune serum could neutralize the toxic substances produced by the diphtheria pathogen, *Corynebacterium diphtheriae*. Such a neutralizing substance, which is called an antitoxin, is a specific antibody produced by a host as it responds to a bacterial exotoxin or its corresponding toxoid (inactivated toxin). The antitoxin combines with the exotoxin to neutralize it (Figure 18.9a). Antitoxins produced in an animal can be injected into humans to provide passive immunity against a toxin. Antitoxins from horses are routinely used to prevent or treat diphtheria and botulism; tetanus antitoxin is usually of human origin.

These therapeutic uses of neutralization reactions have led to their use as diagnostic tests. Viruses that exhibit their cytopathic (cell-damaging) effects in cell culture or embryonated eggs can be used to detect the presence of neutralizing viral antibodies (see page 441). If the serum to be tested contains antibodies against the particular virus, the antibodies will prevent that virus from infecting cells in the cell culture or eggs, and no cytopathic effects will be seen. Such tests, known as in vitro neutralization tests, can thus be used both to identify a virus and to ascertain the viral antibody titer. In vitro neutralization tests are comparatively complex to carry out and are becoming less common in modern clinical laboratories.

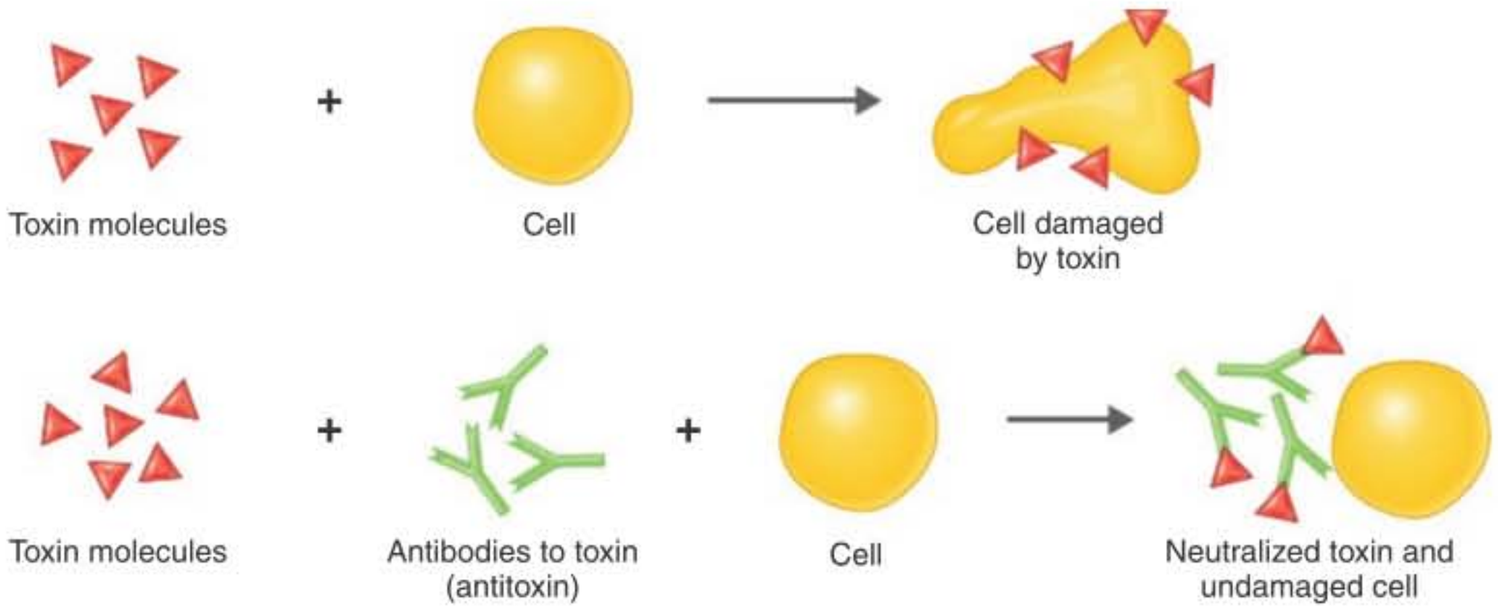
A neutralization test used mostly for the serological typing of viruses is the **viral hemagglutination inhibition test.** Certain viruses such as those causing influenza, mumps, and measles have surface proteins that will cause the agglutination of red blood cells. This test finds its most common use in the subtyping of influenza viruses, although more laboratories are likely to be familiar with ELISA tests for this purpose. If a person's serum contains antibodies against these viruses, these antibodies will react with the viruses and neutralize them (Figure 18.9b). For example, if hemagglutination occurs in a mixture of measles virus and red blood cells but does not occur when the patient's serum is added to the mixture, this result indicates that the serum contains antibodies that have bound to and neutralized the measles virus.

CHECK YOUR UNDERSTANDING

- In what way is there a connection between hemagglutination and certain viruses? 18-14
- Which of these tests is an antigen–antibody reaction: precipitation or viral hemagglutination inhibition? 18-15

Complement-Fixation Reactions

In Chapter 16 (pages 463–468), we discussed a group of serum proteins collectively called complement. During most antigen–antibody reactions, complement binds to the antigen–antibody complex and is used up, or fixed. This process of **complement fixation** can be used to detect very small amounts of antibody. Antibodies that do not produce a visible reaction, such as precipitation or agglutination, can be demonstrated by the fixing of complement during the



(a) The effects of a toxin on a susceptible cell and neutralization of the toxin by antitoxin

(b) Viral hemagglutination test to detect antibodies to a virus. These viruses will normally cause hemagglutination when mixed with red blood cells. If antibodies to the virus are present, as shown here, they neutralize and inhibit hemagglutination.

Figure 18.9 Reactions in neutralization tests.

Q Why does hemagglutination indicate that a patient does not have a specific disease?

antigen–antibody reaction. Complement fixation was once used in the diagnosis of syphilis (Wassermann test) and is still used to diagnose certain viral, fungal, and rickettsial diseases. The complement-fixation test requires great care and good controls, one reason the trend is to replace it with newer, simpler tests. The test is performed in two stages: complement fixation and indicator (Figure 18.10).

CHECK YOUR UNDERSTANDING

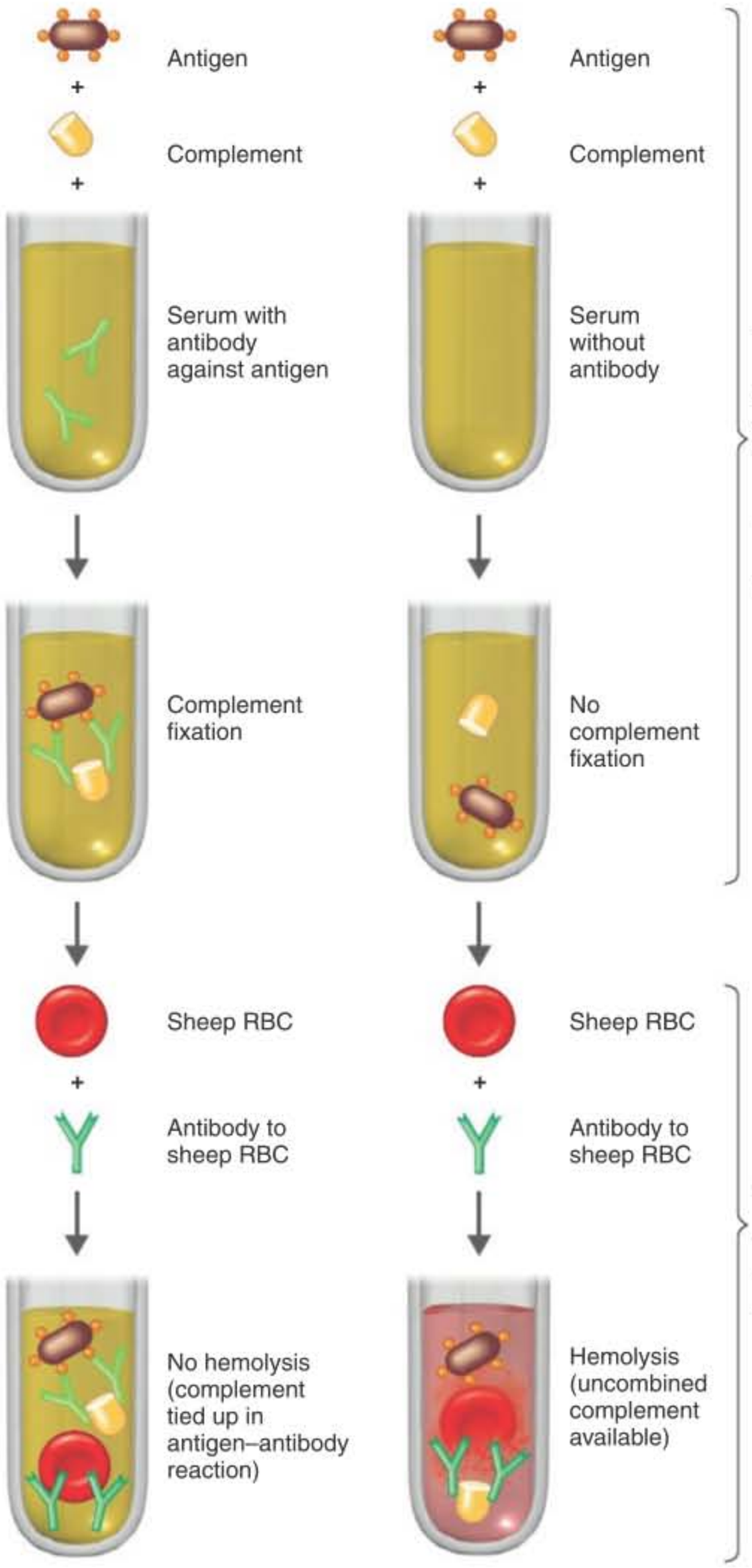
✓ Why is complement given its name? 18-16

Fluorescent-Antibody Techniques

Fluorescent-antibody (FA) techniques can identify microorganisms in clinical specimens and can detect the presence of a specific antibody in serum (Figure 18.11). These techniques combine fluorescent dyes such as fluorescein isothiocyanate (FITC) with antibodies to make them fluoresce when exposed to ultraviolet light (see Figure 3.6, page 61). These procedures are quick, sensitive, and very specific; the FA test for rabies can be performed in a few hours and has an accuracy rate close to 100%.

Q&A Fluorescent-antibody tests are of two types, direct and indirect. **Direct FA tests** are usually used to identify a microorganism in a clinical specimen (Figure 18.11a). During this procedure, the specimen containing the antigen to be identified is fixed onto a slide. Fluorescein-labeled antibodies are then added, and the slide is incubated briefly. Next the slide is washed to remove any antibody not bound to antigen and is then examined under the fluorescence microscope for yellow-green fluorescence. The residual antibody will be visible even if the antigen, such as a virus, is submicroscopic in size.

Indirect FA tests are used to detect the presence of a specific antibody in serum following exposure to a microorganism (Figure 18.11b). They are often more sensitive than direct tests. During this procedure, a known antigen is fixed onto a slide. The test serum is then added, and, if antibody that is specific to that microbe is present, it reacts with the antigen to form a bound complex. For the antigen–antibody complex to be seen, fluorescein-labeled **antihuman immune serum globulin (anti-HISG),** an antibody that reacts specifically with *any* human antibody, is added to the slide. Anti-HISG will be present only if the specific antibody has reacted with its antigen and is therefore present as well. After the slide has been incubated and washed



(a) Positive test. All available complement is fixed by the antigen–antibody reaction; no hemolysis occurs, so the test is positive for the presence of antibodies.

(b) Negative test. No antigen–antibody reaction occurs. The complement remains, and the red blood cells are lysed in the indicator stage, so the test is negative.

Figure 18.10 The complement-fixation test. This test is used to indicate the presence of antibodies to a known antigen. Complement will combine (be fixed) with an antibody that is reacting with an antigen. If all the complement is fixed in the complement-fixation stage, then none will remain to cause hemolysis of the red blood cells in the indicator stage.

Q Why does red blood cell lysis indicate that the patient does not have a specific disease?

(to remove unbound antibody), it is examined under a fluorescence microscope. If the known antigen fixed to the slide appears fluorescent, the antibody specific to the test antigen is present.

An especially interesting adaptation of fluorescent antibodies is the **fluorescence-activated cell sorter (FACS).** In Chapter 17, we learned that T cells carry antigenically specific molecules such as CD4 and CD8 on their surface, and these are characteristic of certain groups of T cells. The depletion of $CD4^+$ T cells is used to follow the progression of AIDS; their populations can be determined with a FACS.

The FACS is a modification of a *flow cytometer,* in which a suspension of cells leaves a nozzle as droplets containing no more than one cell each (see page 288). A laser beam strikes each cell-containing droplet and is then received by a detector that determines certain characteristics such as size (**Figure 18.12**). If the cells carry FA markers to identify them as $CD4^+$ or $CD8^+$ T cells, the detector can measure this fluorescence. As the laser beam detects a cell of a preselected size or fluorescence, an electrical charge, either positive or negative, can be imparted to it. As the charged droplet falls between electrically charged plates, it is attracted to one receiving tube or another, effectively separating cells of different types. Millions of cells can be separated in an hour with this process, all under sterile conditions, which allows them to be used in experimental work.

An interesting application of the flow cytometer is sorting sperm cells to separate male (Y-carrying) and female (X-carrying) sperm. The female sperm (meaning that it will result in a female embryo when it fertilizes the egg) contains more DNA, 2.8% more in humans, 4% in animals. When the sperm is stained with a fluorescent dye specific for DNA, the female sperm glows more brightly when illuminated by the laser beam because it has more DNA and therefore can be separated out. The technique was developed for agricultural purposes. However, it has received medical approval for use in humans where couples carry genes for inherited diseases that affect only boys.

CHECK YOUR UNDERSTANDING

✓ Which test is used to detect antibodies against a pathogen: the direct or the indirect fluorescent-antibody test? **18-17**

Enzyme-Linked Immunosorbent Assay (ELISA)

The **enzyme-linked immunosorbent assay (ELISA)** is the most widely used of a group of tests known as *enzyme immunoassay (EIA).* There are two basic methods. The *direct ELISA* detects antigens, and the *indirect ELISA* detects antibodies. A microtiter plate with numerous shallow wells is used in both procedures (see Figure 10.11a, page 288). Variations of the test exist; for example, the reagents can be bound to tiny latex particles rather than to the surfaces of the microtiter plates. ELISA procedures are popular primarily because they require little interpretive skill to read; the results tend to be clearly positive or clearly negative.

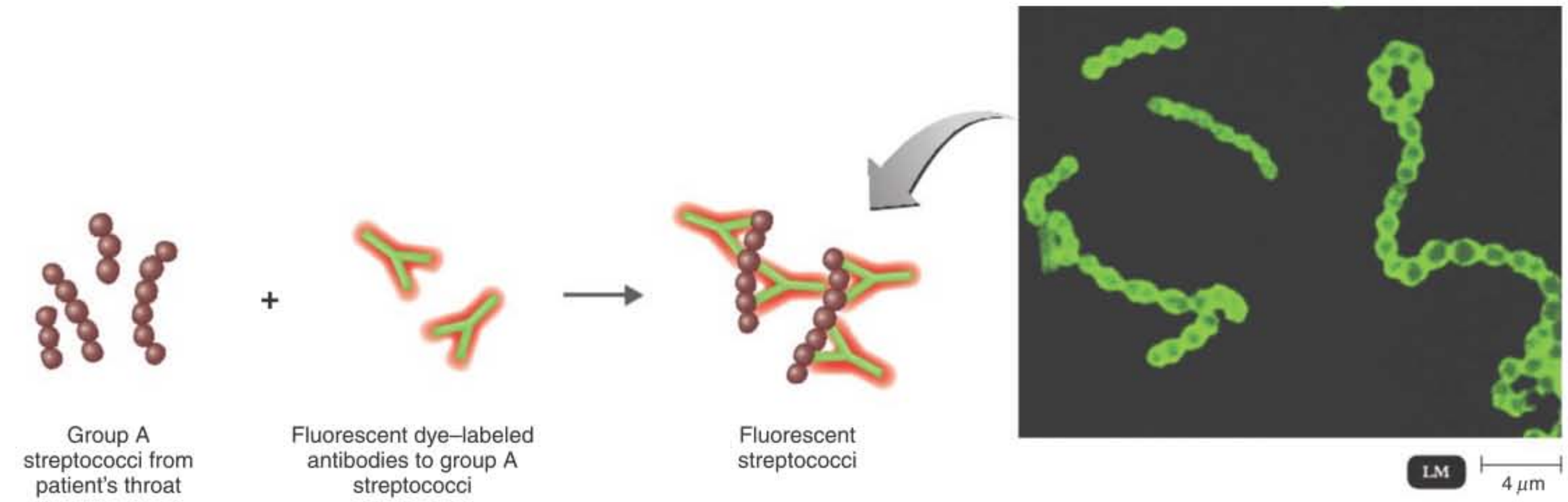


Figure 18.11 Fluorescent-antibody (FA) techniques. (**a**) A direct FA test to identify group A streptococci. (**b**) In an indirect FA test such as that used in the diagnosis of syphilis, the fluorescent dye is attached to antihuman immune serum globulin, which reacts with any human immunoglobulin (such as the *Treponema pallidum*–specific antibody) that has previously reacted with the antigen. The reaction is viewed through a fluorescence microscope, and the antigen with which the dye-tagged antibody has reacted fluoresces (glows) in the ultraviolet illumination.

Q **Differentiate a direct from an indirect FA test.**

Many ELISA tests are available for clinical use in the form of commercially prepared kits. Procedures are often highly automated, with the results read by a scanner and printed out by computer (see Figure 10.11, page 288). Some tests based on this principle are also available for use by the public; one example is a commonly available home pregnancy test (**Figure 18.13**).*

*An Egyptian papyrus dating to 1350 B.C. described a pregnancy test in which a woman would urinate on wheat and barley seeds for several days. Growth of the seeds was an indication of pregnancy. In 1963 this theory was tested, and 70% of the time the urine of a pregnant woman did indeed promote growth whereas the urine of men or nonpregnant women did not. Elevated levels of estrogens were considered a likely cause. (The use of two seed species was intended to determine gender: barley, male; wheat, female).

Direct ELISA

The direct ELISA method is shown in **Figure 18.14a** (page 518). A common use of the direct ELISA test is to detect the presence of drugs in urine. For these tests, antibodies specific for the drug are adsorbed to the well on the microtiter plate. (The availability of monoclonal antibodies has been essential to the widespread use of the ELISA test.) When the patient's urine sample is added to the well, any of the drug that it contained would bind to the antibody and is captured. The well is rinsed to remove any unbound drug. To make a visible test, more antibodies specific to the drug are now added (these antibodies have an enzyme attached to them—therefore, the term *enzyme-linked*) and will react with the already-captured drug, forming a "sandwich" of antibody/drug/enzyme-linked antibody. This positive test can be

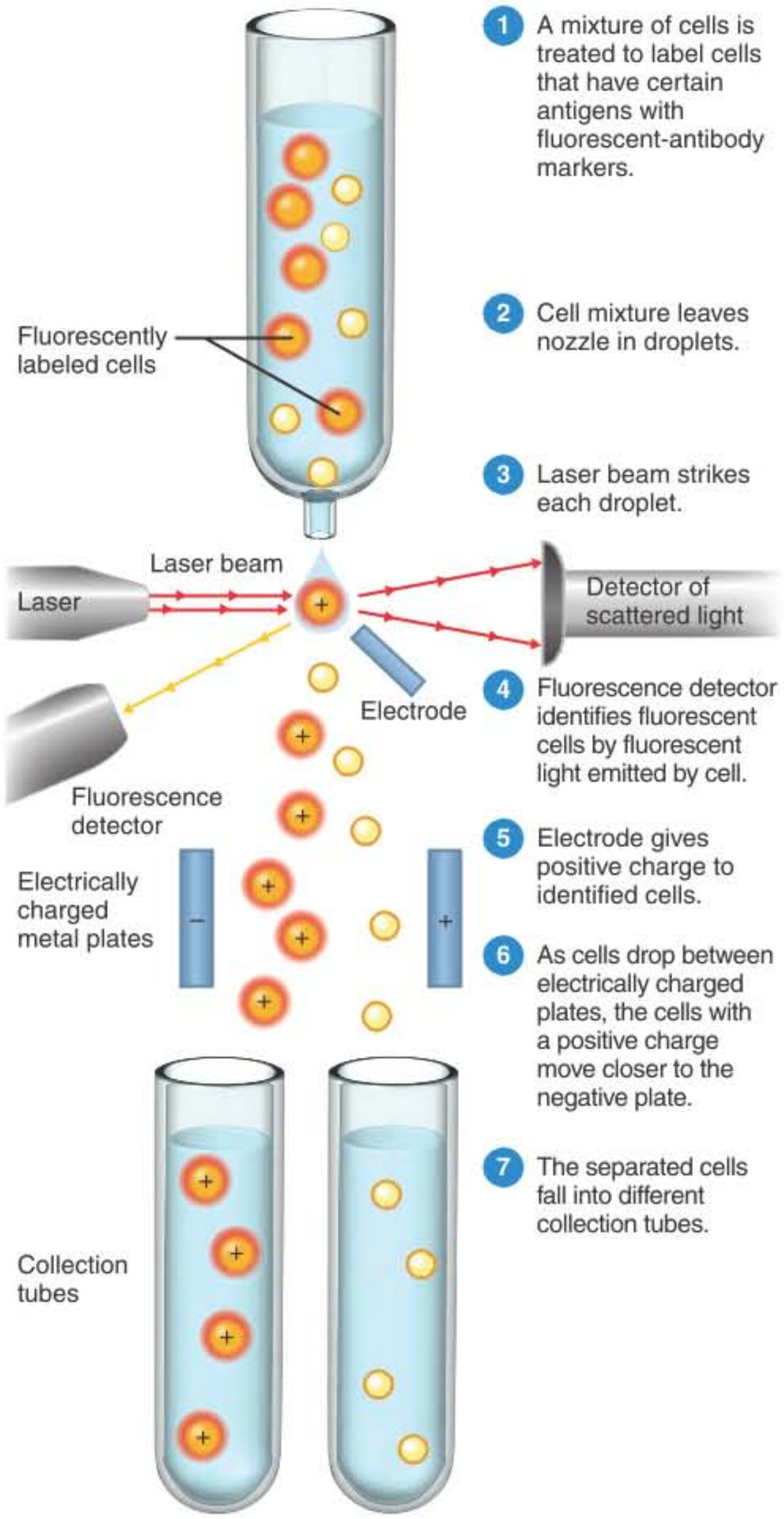


Figure 18.12 The fluorescence-activated cell sorter (FACS). This technique can be used to separate different classes of T cells. A fluorescence-labeled antibody reacts with, for example, the CD4 molecule on a T cell.

Q Provide an application of FACS to follow the progress of HIV infection.

detected by adding a substrate for the linked enzyme; a visible color is produced by the enzyme reacting with its substrate.

Indirect ELISA

The indirect ELISA text, illustrated in **Figure 18.14b**, detects antibodies in a patient's sample rather than an antigen such as a drug. Indirect ELISA tests are used, for example, to screen blood for antibodies to HIV (see page 545). For such a purpose, the microtiter well contains an antigen, such as the inactivated virus that causes the disease the test is designed to diagnose. A sample of the patient's blood is added to the well; if it contains antibodies against the virus, they will react with the virus. The well is rinsed to remove unbound antibodies. If antibodies in the blood and the virus in the well have attached to each other, they will remain in the well—a positive test. To make a positive test visible, some anti-HISG (an immunoglobulin that will attach to *any* antibody, including the one in the patient's serum that has attached to the virus in the well; see page 513) is added. The anti-HISG is linked to an enzyme. A positive test consists of a "sandwich" or a virus/antibody/enzyme-linked-anti-HISG. At this point, the substrate for the enzyme is added, and a positive test is detected by the color change caused by the enzyme linked to the anti-HISG.

Western Blotting (Immunoblotting)

Western blotting, often simply called *immunoblotting*, can be used to identify a specific protein in a mixture. When this specific protein is an antibody, the technique is valuable in diagnosing disease. The components of the mixture are separated by electrophoresis in a gel and then transferred to a protein-binding sheet (blotter). There the protein/antigen is flooded with an enzyme-linked antibody. The location of the antigen and the enzyme-linked antibody reactant can be visualized, usually with a color-reacting label similar to an ELISA test reaction (see Figure 18.14). The most frequent application is in a confirmatory test for HIV infection (see page 545). Figure 10.12 on page 289 illustrates the procedure.*

CHECK YOUR UNDERSTANDING

- ✓ Which test is used to detect antibodies against a pathogen, the direct or the indirect ELISA test? **18-18**
- ✓ How are antibodies detected in Western blotting? **18-19**

The Future of Diagnostic and Therapeutic Immunology

The introduction of monoclonal antibodies has revolutionized diagnostic immunology by making available large, economical amounts of specific antibodies. This has led to many newer diagnostic tests that are more sensitive, specific, rapid, and simpler to use. For example, tests to diagnose sexually transmitted chlamydial infections and certain protozoan-caused intestinal parasitic diseases are coming into common use. These tests had previously required relatively difficult culture or microscopic methods for diagnosis. At the same time, the use of many of the classic serological tests, such as complement-fixation tests, is declining.

*The naming of Western blotting is a bit of scientific whimsy. The Southern blotting procedure used for detecting DNA fragments (page 262), which was named for the inventor Ed Southern, led to a similar procedure for detecting mRNA fragments being named Northern blotting. This "directional system" was continued when a new blotting procedure for identifying proteins was developed—hence, Western blotting.

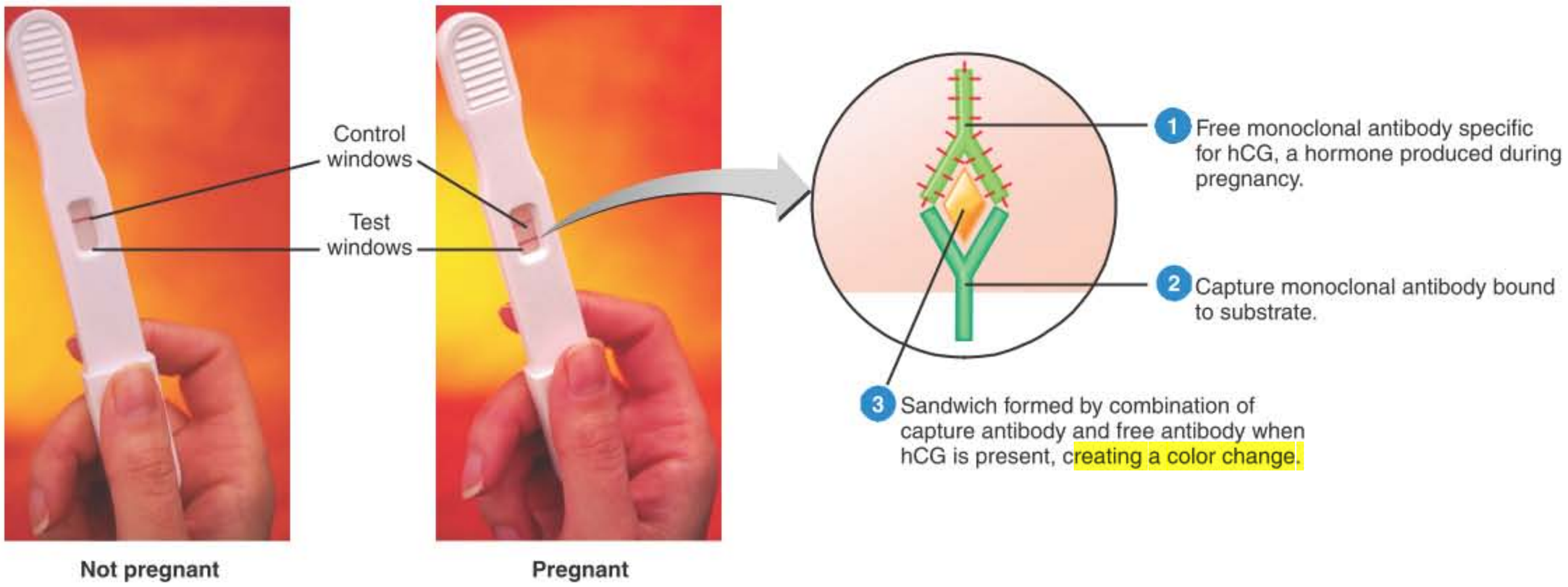


Figure 18.13 The use of monoclonal antibodies in a home pregnancy test. Home pregnancy tests detect a hormone called human chorionic gonadotropin (hCG) that is excreted only in the urine of a pregnant woman.

Q What is the antigen in the home pregnancy test?

Most newer tests will require less human judgment to read, and they require fewer highly trained personnel.

The use of certain *nonimmunological* tests, such as the PCR and DNA probes that were discussed in Chapter 10 (page 292), is increasing. Some of these tests will become automated to a significant degree. For example, a DNA chip (see Figure 10.17, page 293) containing over 50,000 DNA probes for genetic information expected in possible pathogens can be exposed to a test sample. This chip is scanned and its data automatically analyzed. PCR tests are also becoming highly automated.

Most of the diagnostic tests described in this chapter are those used in the developed world, where laboratory budgets are lavish compared to the funds available in much of the world. In many countries the money available for all medical expenditures, for diagnosis and treatment alike, is tragically small.

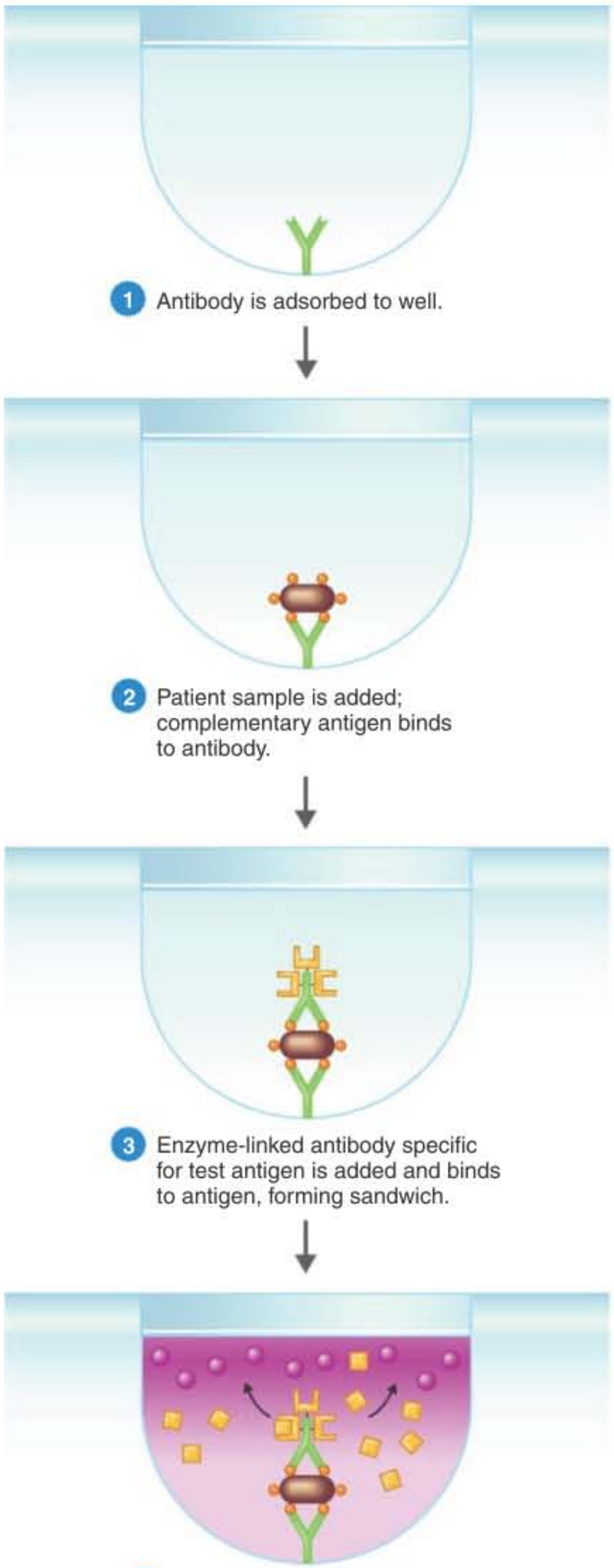
The diseases that most of these diagnostic methods target are also those that are more likely to be found in developed countries. In many parts of the world, especially tropical Africa and tropical Asia, there is an urgent need for diagnostic tests for diseases endemic in those areas, such as malaria, leishmaniasis, AIDS, Chagas' disease, and tuberculosis. These tests will need to be inexpensive and simple enough to be carried out by personnel with minimal training.

The tests described in this chapter are most often used to detect existing disease. In the future, diagnostic testing will probably also be directed at *preventing* disease. In the United States we regularly see reports of outbreaks of foodborne disease. Sampling methods that would allow complete identification, (including specific pathogenic serovars), within a few hours, or even minutes, would be a vast saving in time. Such rapid diagnostic tests would be especially valuable for tracking outbreaks of infectious disease carried by fruits and vegetables, such as the Salmonella outbreak in the summer of 2008. These products do not have the identifying marks that facilitate the tracking of packaged food products. This saving in time would be translated into immense economic savings for growers and retailers. It would also lead to less human illness and, perhaps, a saving of lives.

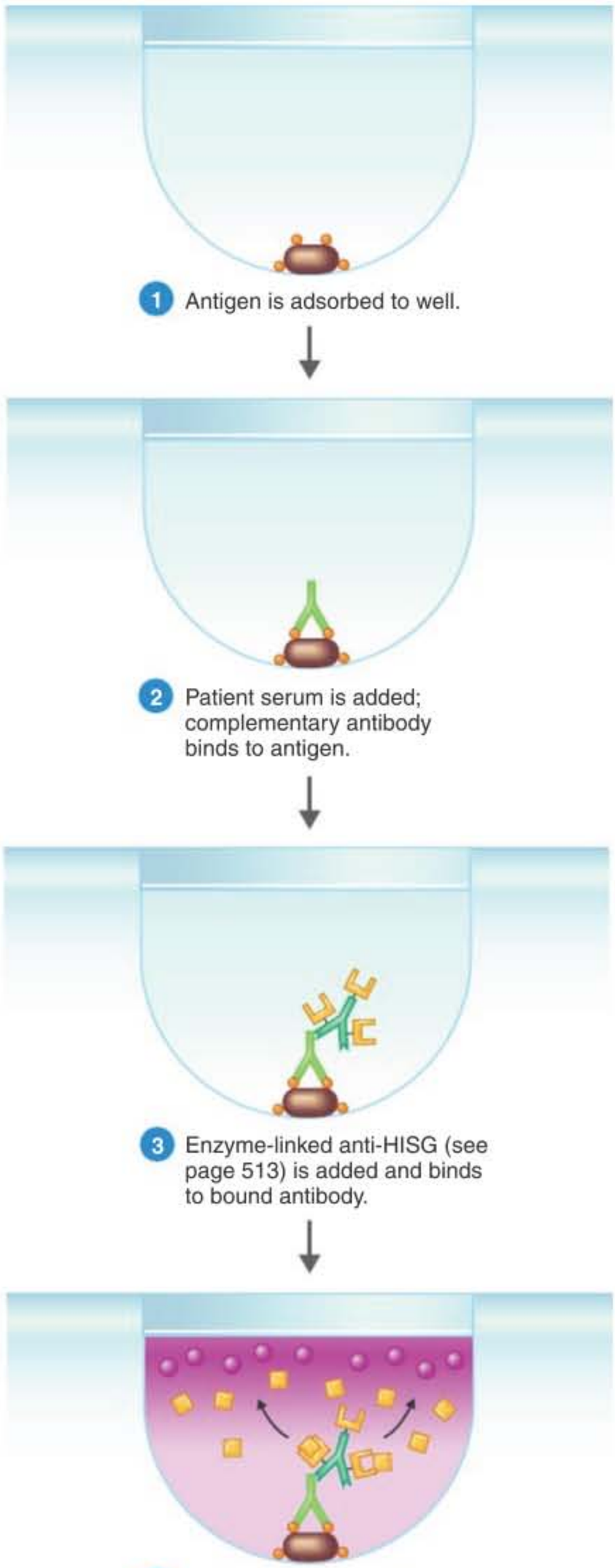
Not every topic discussed in this chapter is necessarily directed at the detection and prevention of disease. As was mentioned on page 509, Mabs have applications in the therapy of disease as well. These are already in use to treat certain cancers such as breast cancer and non-Hodgkin's lymphoma, as well as inflammatory diseases such as rheumatoid arthritis. Currently, Mabs are being tested for many disease conditions; these include asthma, sepsis, coronary artery disease, and several viral infections. They are also being studied as a way to treat the immune-caused neurological disease, multiple sclerosis.

CHECK YOUR UNDERSTANDING

- How has the development of monoclonal antibodies revolutionized diagnostic immunology? **18–20**



(a) A positive direct ELISA to detect antigens



(b) A positive indirect ELISA to detect antibodies

Figure 18.14 The ELISA method. The components are usually contained in small wells of a microtiter plate. For an illustration of a technician carrying out an ELISA test on such a microtiter plate and the use of a computer to read the results, see Figure 10.11 on page 288.

Q **Differentiate a direct from an indirect ELISA test.**