A 13-month-old girl is brought into the hospital, coughing and crying. Her mother tells the pediatrician that she had noticed a mucous discharge pooling in the corners of her child’s eyes the previous evening. The pediatrician checks the girl’s medical record and notes that she has not yet received her first measles/mumps/rubella (MMR) vaccination. The doctor then swabs the child’s throat, arranges for a blood sample to be taken, and tells the mother that they are going to have the lab perform a “special immune test” to confirm the diagnosis: measles. Because the pediatrician has seen several children with measles in the past few weeks and the child’s symptoms support the diagnosis, a nurse administers an intramuscular injection of immunoglobulin against measles. The nurse explains that this injection will protect the child against the most severe form of the illness, which can be fatal. The physician also schedules the daughter for a routine measles/mumps/rubella immunization. Mother and daughter return home to rest and recuperate.

How do immunoglobulins and vaccines work? How do immunological tests aid in the diagnosis of specific diseases? This chapter is an introduction to the important topics of immunization and immune testing.
In this chapter we will discuss three applications of immunology: active immunization (vaccination); passive immunotherapy using immunoglobulins (antibodies); and immune testing. Vaccination has proven the most efficient and cost-effective method of controlling infectious diseases. Without the use of effective vaccines, millions more people worldwide would suffer each year from potentially fatal infectious diseases, including measles, mumps, and polio. The administration of immunoglobulins has further reduced morbidity and mortality from certain infectious diseases, such as hepatitis A and yellow fever, in unvaccinated individuals. Medical personnel also make practical use of the immune response as a diagnostic procedure. For example, the detection of antibodies to HIV in a person’s blood indicates that the individual has been exposed to that virus and may develop AIDS. The remarkable specificity of antibodies also enables the detection of drugs in urine, recognition of pregnancy at early stages, and the identification or characterization of other biological material. The many tests developed for these purposes are the focus of the discipline of serology and are discussed in the second half of this chapter.

Immunization

As we saw in Chapter 16, an individual may be made immune to an infectious disease by two artificial methods: active immunization, which involves administering antigens to a patient so that the patient actively mounts an adaptive immune response, and passive immunotherapy, in which a patient acquires temporary immunity through the transfer of antibodies formed by other individuals or animals.

In the following sections we will review the history of immunization before examining immunization and immunotherapy in more detail.

Brief History of Immunization

Learning Objective

✔ Discuss the history of vaccination from the 12th century through the present.

As early as the 12th century, the Chinese noticed that children who recovered from smallpox never contracted the disease a second time. They therefore adopted a policy of deliberately infecting young children with particles of ground smallpox scabs from children who had survived mild cases. By doing so, they succeeded in significantly reducing the population’s overall morbidity and mortality from the disease. News of this procedure, called variolation (var’e-ō-la’shən), spread westward through central Asia, and the technique was widely adopted.

Lady Mary Montagu (1689–1762), the wife of the English ambassador to the Ottoman Empire, learned of the procedure, had it performed on her own children, and told others about it upon her return to England in 1721. As a result, variolation came into use in England and in the American colonies. Although effective and usually successful, variolation caused death from smallpox in 1–2% of recipients and in people exposed to recipients, so in time the procedure was outlawed.

Thus, when the English physician Edward Jenner demonstrated in 1796 that protection against smallpox could be conferred by inoculation with crusts from a person infected with cowpox—a related but very mild disease—the new technique was adopted. Because cowpox was also called vaccinia (vak’sin’e-ə), Jenner called the new technique vaccination (vak’si-nə’shən), and the protective inoculum a vaccine (vak-se’n). Today we use the term immunization to refer to the administration of any antigenic inoculum, which are all called vaccines. For many years thereafter, vaccination against smallpox was widely practiced, even though no one understood how it worked or whether similar techniques could protect against other diseases.

In 1879, Louis Pasteur conducted experiments on the bacterium Pasteurella multocida (pas-ter’-ə-lə mul-tō’si-da) and demonstrated that he could make an effective vaccine against this organism (which causes a disease in birds called fowl cholera). Once the basic principle of vaccine manufacture was understood, vaccines against anthrax and rabies rapidly followed. After it was discovered that these vaccines provide protection through the actions of antibodies, the technique of transferring protective antibodies to susceptible individuals—that is, passive immunotherapy (im’u-ňə-thər-a’-pe) —was developed soon thereafter.

By the late 1900s, immunologists and health care providers had formulated vaccines that significantly reduced the number of cases of many infectious diseases (Figure 17.1). We also have successful vaccines against some types of cancer. Health care providers, governments, and international organizations working together have rid the world of naturally occurring smallpox, and we hope for the worldwide eradication of polio, measles, mumps, and rubella. Highlight: Why Isn’t There a Cold Vaccine? p. 492 discusses why a vaccine for the common cold does not yet exist.

Even though immunologists have produced vaccines that protect people against many deadly diseases, a variety of political, social, economic, and scientific problems prevent vaccines from reaching all those who need them. In developing nations worldwide, over 3 million children still die each year from vaccine-preventable infectious diseases, primarily because of political obstacles. Additionally, some pathogens, such as the protozoa of malaria and the virus of AIDS, still frustrate attempts to develop effective vaccines against them. Furthermore, the existence of vaccine-associated risks—both medical risks (the low but persistent incidence of vaccine-caused diseases) and financial risks (the high costs of developing and producing vaccines, and the risk of lawsuits by vaccine recipients who have adverse reactions)—has in recent years discouraged investment in new vaccines. Thus, although the history of immunization is marked by stunning advancements in public health, the future of immunization poses immense challenges.

Next we take a closer look at active immunization, commonly known as vaccination.

1From Latin vacca, meaning cow.
WHY ISN'T THERE A COLD VACCINE?

We have vaccines for the flu, so why don't we have a vaccine for the common cold? The reason is that whereas strains of only one influenza virus cause flu, over 200 different adenoviruses, coronaviruses, and rhinoviruses are known to cause the common cold, and each of these viruses has its own distinct antigens and antigenic strains, making it extremely difficult to create a single vaccine to prevent them all. To further complicate matters, viruses can mutate, resulting in changes in their antigens; with over 200 different cold viruses in existence, such mutations create immense logistical challenges in vaccine development. Fortunately, the common cold typically lasts only a few days and is adequately treated with rest and self-care.

The general types of vaccines, each of which has its own combination of strengths and weaknesses, are attenuated (live) vaccines, killed (or inactivated) vaccines, toxoid vaccines, combination vaccines, and recombinant gene vaccines. Each of these is named for the type of antigen used in the inoculum.

**Attenuated (Live) Vaccines** Virulent microbes are normally not used in vaccines, because they cause disease. Instead, immunologists reduce virulence so that, although still active, the pathogens no longer cause disease. The process of reducing virulence is called **attenuation** (a-ten-a-shun). The most common method for attenuating viruses involves raising them for numerous generations in tissue culture cells until the viruses lose the ability to produce disease. For example, rabies viruses, which preferentially attack nerve cells, are subjected to prolonged tissue culture until their virulence to nerve cells is lost; the resulting avirulent viruses can be used in vaccines. Bacteria may be made avirulent by culturing them under unusual conditions or by using genetic manipulation.

**Attenuated vaccines**—those containing attenuated microbes—are also called modified live vaccines. Because they contain active but avirulent organisms or viruses, these vaccines cause very mild infections but no serious disease under normal conditions. Attenuated viruses in such a vaccine infect host cells and replicate; the infected cells then process endogenous viral antigens. As a result, modified live viral vaccines trigger a cell-mediated immune response dominated by type 1 helper T cells (Th1) and cytotoxic T cells (Tc). Because modified live vaccines contain active microbes, a large number of antigen molecules are available to stimulate an immune response. Further, vaccinated individuals can infect those around them, providing contact
immunity—that is, immunity beyond the individual receiving the vaccine.

Although usually very effective, attenuated vaccines can be hazardous because modified microbes may retain enough residual virulence to cause disease in immunosuppressed people. Pregnant women should not receive live vaccines because of the danger that the attenuated pathogen will cross the placenta and harm the fetus. Occasionally, modified viruses actually revert to wild type or mutate to a form that causes persistent infection or disease. For example, in 2000 a polio epidemic in the Dominican Republic and Haiti resulted from the reversion of an attenuated live virus in oral polio vaccine to a virulent poliovirus. For this reason, we no longer use live polio vaccine to immunize children in the United States.

Health care providers and government agencies must carefully balance the benefits of attenuated vaccines against their risks. As a result, they may change their recommended immunization schedules.

**Inactivated (Killed) Vaccines** For some diseases, live vaccines have been replaced by inactivated vaccines, which are of two types: whole agent vaccines are produced with deactivated but whole microbes, whereas subunit vaccines are produced with antigenic fragments of microbes. Because neither whole agent nor subunit vaccines can replicate, revert, mutate, or retain residual virulence, they are safer than live vaccines. However, because they cannot replicate, several "booster" doses must be administered to achieve full immunity, and immunized individuals do not stimulate contact immunity. Also, with whole agent vaccines, nonantigenic portions of the microbe occasionally stimulate a painful inflammatory response in some individuals. As a result, whole agent pertussis vaccine is now being replaced with a subunit vaccine called acellular pertussis vaccine.

When microbes are killed for use in vaccines, it is important that their antigens remain as similar to those of living organisms as possible. If chemicals are used for killing, they must not alter the antigens responsible for stimulating protective immunity. A commonly used inactivating agent is formaldehyde, which denatures proteins and nucleic acids.

Because the microbes of inactivated vaccines cannot reproduce, they do not present as many antigenic molecules to the body as do live vaccines; therefore, inactivated vaccines are antigenically weak. They are administered in high doses or in multiple doses, or incorporated with materials called adjuvants (ad'joo-vantz), substances that increase the effective antigenicity of the vaccine by stimulating Toll-like receptors and their actions. Unfortunately, high individual doses and multiple dosing increase the risk of producing allergies, and the use of adjuvants to increase antigenicity may stimulate local inflammation. Table 17.1 lists some common adjuvants and their effects in enhancing the efficacy of vaccines.

Because all types of killed vaccines are recognized by the immune system as exogenous antigens, they stimulate an antibody immune response.

**Toxoid Vaccines** For some bacterial diseases, notably tetanus and diphtheria, it is more efficacious to induce an immune response against toxins than against cellular antigens. Toxoid (tok'soyd) vaccines are chemically or thermally modified toxins that are used in vaccines to stimulate active immunity. As with killed vaccines, toxoids stimulate antibody-mediated immunity. Because toxoids have few antigenic determinants, effective immunization requires multiple childhood doses as well as reinforcement every 10 years for life. ANIMATIONS: Vaccines: Function, Types

**Combination Vaccines** The Centers for Disease Control and Prevention (CDC) has approved several combination vaccines for routine use. These vaccines combine antigens from several toxoids and inactivated pathogens that are administered simultaneously. Examples include MMR—vaccine against measles, mumps, and rubella—and Pentacel, which is a vaccine against diphtheria, tetanus, pertussis (whooping cough), polio, and diseases of Haemophilus influenzae (hē-mof'i-lüs in-flu-en'zı).

**Vaccines Using Recombinant Gene Technology** Although live, inactivated, and toxoid vaccines have been highly successful in controlling infectious diseases, researchers are always seeking ways to make vaccines more effective, cheaper, and safer and to make new vaccines against pathogens that have been difficult to protect against. For example, scientists have developed a recombinant DNA vaccine against a fungus, Blastomyces (blas-tô-mi'séz)—the first vaccine against a fungal pathogen. Scientists can also use a variety of genetic recombinant techniques to make improved vaccines. For example, they can selectively delete virulence genes from a pathogen, producing an irreversibly attenuated microbe, one that cannot revert to a virulent pathogen (Figure 17.2a).

### Table 17.1 Some Common Adjuvants

<table>
<thead>
<tr>
<th>Adjuvant</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum phosphate (alum)</td>
<td>Slows processing and degradation of antigen</td>
</tr>
<tr>
<td>Saponin (soaplike plant product)*</td>
<td>Stimulates T cell responses</td>
</tr>
<tr>
<td>Mineral oil</td>
<td>Slows processing and degradation of antigen</td>
</tr>
<tr>
<td>Freund's complete adjuvant (mineral oil containing killed mycobacteria)*</td>
<td>Slows processing and degradation of antigen; stimulates T cell responses</td>
</tr>
</tbody>
</table>

*Considered too toxic for humans; used in vaccinating animals only.
Figure 17.2 Some uses of recombinant DNA technology for making improved vaccines. (a) Deletion of virulence gene(s) to create an attenuated pathogen for use in a vaccine. (b) Insertion of gene that codes for a selected antigenic protein into a cell, which then produces large quantities of the antigen for use in a vaccine. (c) Insertion of a gene that codes for a selected antigenic protein into a cell or virus, which displays the antigen. The entire recombinant is used in a vaccine. (d) Injection of DNA containing a selected gene (in this case, as part of a plasmid) into an individual. Once some of this DNA is incorporated into the genome of a patient's cells, those cells synthesize and process the antigen, which stimulates a cell-mediated immune response.
Scientists also use recombinant techniques to produce large quantities of very pure viral or bacterial antigens for use in vaccines. In this process, scientists isolate the gene that codes for an antigen and insert it into a bacterium, yeast, or other cell, which then expresses the antigen (Figure 17.2c). Vaccine manufacturers produce hepatitis B vaccine in this manner using recombinant yeast cells.

Alternatively, a genetically altered microbial cell or virus may itself be used as a live recombinant vaccine (Figure 17.2b). Experimental recombinant vaccines of this type have used adenoviruses, herpesviruses, poxviruses, and bacteria such as *Salmonella* (sal'mo-nel'a). Vaccinia virus (a poxvirus) is used because it is easy to administer by dermal scratching or orally, and because its large genome makes inserting a new gene into it relatively easy.

Another innovative method of immunization involves injection not of antigens but instead of the DNA that codes for the antigen. For example, the DNA coding for a pathogen’s antigen can be inserted into a plasmid vector, which is then injected into the body (Figure 17.2d). The body’s cells then transcribe and translate the gene to produce antigen, which triggers a cell-mediated immune response.

### Vaccine Manufacture

Manufacturers mass-produce many vaccines by growing microbes in laboratory culture vessels, but because viruses require a host cell to reproduce, they are cultured inside chicken eggs. Availability of sterile eggs is thus critical for manufacturing viral vaccines such as flu vaccines. Because the vaccines are produced in eggs, physicians must withhold such immunizations from patients with egg allergies. Research on gene-based vaccines and development of vaccines in genetically modified plants may result in safer vaccines.

### Recommended Immunizations

The CDC and medical associations publish recommended immunization schedules for children, adults, and special populations such as health care workers and HIV-positive individuals. The recommendations are frequently modified to reflect changes in the relationships between pathogens and the human population. Figure 17.3 highlights the general 2009 immunization schedules recommended by the CDC. In Table 17.2 on p. 497, facts concerning the types of vaccines available for immunizing against each of the diseases in the vaccination schedule as well as some other available vaccines are listed. Vaccines against anthrax, cholera, plague, tuberculosis, and other diseases are available, but the CDC does not recommend them for the general U.S. population.

It is important that patients follow the recommended immunization schedule not only to protect themselves but also to provide society with herd immunity. Herd immunity is the protection provided all individuals in a population due to the inability of a pathogen to effectively spread when a large proportion of individuals (typically more than 75%) are resistant. When immunization compliance in a population has fallen, local epidemics have resulted.

### Vaccine Safety

Health care providers must carefully weigh the risks associated with vaccines against their benefits. A common vaccine-associated problem is mild toxicity. Some vaccines—especially whole agent vaccines that contain adjuvants—may cause pain at the injection site for several hours or days after injection. In rare cases, toxicity may result in general malaise and possibly a fever high enough to induce seizures. Although not usually life threatening, the potential for these symptoms may be sufficient to discourage people from being immunized or having their infants immunized.

A much more severe problem associated with immunization is the risk of anaphylactic shock, an allergic reaction, which may develop to some component of the vaccine, such as egg proteins, adjuvants, or preservatives. Because people are rarely aware of such allergies ahead of time, recipients should remain for several minutes in the physician’s office, where epinephrine is readily available to counter any signs of an allergic reaction.

A third major problem associated with immunization is that of residual virulence, which we previously discussed. Attenuated viruses occasionally cause disease, not only in fetuses and immunosuppressed patients but also in healthy children and adults. A good example is the attenuated oral poliovirus vaccine (OPV), which was commonly used in the United States until the late 1990s. Though a very effective vaccine, it causes clinical poliomyelitis in 1 of every 2 million recipients or their close contacts. Medical personnel in the United States eliminated this problem by switching to inactivated polio vaccine (IPV).

Over the past two decades, lawsuits in the United States and Europe have alleged that certain vaccines against childhood diseases cause or trigger disorders such as autism, diabetes, and asthma. Extensive research has failed to substantiate these allegations, and to date no conclusive evidence for such a link has been found. Indeed, vaccine manufacturing methods have improved tremendously in recent years, ensuring that modern vaccines are much safer than those in use even a decade ago. The U.S. Food and Drug Administration (FDA) has established a Vaccine Adverse Event Reporting System for monitoring vaccine safety.

The CDC and FDA have determined that the problems associated with immunization are far less serious than the suffering and death that would result if we stopped immunizing people. Beneficial Microbes: Smallpox: To Vaccinate or Not to Vaccinate? on p. 499 discusses the issues surrounding the administration of smallpox vaccinations to the general public.

### Passive Immunotherapy

#### Learning Objectives

- Identify two sources of antibodies for use in passive immunotherapy.
- Compare the relative advantages and disadvantages of active immunization and passive immunotherapy.
Passive immunotherapy (sometimes called passive immunization) involves the administration of preformed antibodies to a patient. Physicians use passive immunotherapy when protection against a recent infection or an ongoing disease is needed quickly. Rapid protection is achieved because passive immunotherapy does not require the body to mount a response; instead, preformed antibodies are immediately available to bind to antigen, enabling neutralization and opsonization to proceed without delay. For example, in a case of botulism poisoning [caused by the toxin of *Clostridium botulinum* (klos-trid’e-ūm bo-tu’li’num)], passive immunotherapy with preformed antibodies against the toxin can prevent death.

Antibodies directed against toxins are also called *antitoxins* (an-të-tok’sinz); *antivenin* used to treat snakebites is an antitoxin. In some cases, infections with certain viruses—hepatitis A and B, measles, rabies, Ebola, chickenpox, and shingles—are treated with antibodies directed against the causative viruses.

For much of the time since passive immunotherapy was first developed, immunologists harvested the desired antibodies from human or animal donors that have either experienced natural
### Principal Vaccines to Prevent Human Diseases

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Disease Agent</th>
<th>Disease</th>
<th>Vaccine Type</th>
<th>Method of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recommended by CDC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>Hepatitis B virus</td>
<td>Hepatitis B</td>
<td>Inactive subunit from recombinant yeast</td>
<td>Intramuscular</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>Rotavirus</td>
<td>Gastroenteritis</td>
<td>Attenuated, recombinant</td>
<td>Oral</td>
</tr>
<tr>
<td>Diphtheria/ tetanus/ acellular pertussis (DTaP)</td>
<td>Diphtheria toxin</td>
<td>Tetanus toxin</td>
<td>Bordetella pertussis</td>
<td>Diphtheria Toxic</td>
</tr>
<tr>
<td>Human papillomavirus (HPV)</td>
<td>Human papillomaviruses</td>
<td>Genital warts, cervical cancer</td>
<td>Inactive recombinant</td>
<td>Intramuscular</td>
</tr>
<tr>
<td>Meningococcal</td>
<td>Neisseria meningitidis</td>
<td>Meningitis</td>
<td>Inactive</td>
<td>Subcutaneous or intramuscular</td>
</tr>
<tr>
<td>Haemophilus influenzae type b (Hib)</td>
<td>Haemophilus influenzae</td>
<td>Meningitis, pneumonia, epiglottitis</td>
<td>Inactivated subunit</td>
<td>Intramuscular</td>
</tr>
<tr>
<td>Pneumococcal (PCV)</td>
<td>Streptococcus pneumoniae</td>
<td>Pneumonia</td>
<td>Inactivated subunit</td>
<td>Intramuscular</td>
</tr>
<tr>
<td>Polio</td>
<td>Poliovirus</td>
<td>Poliomyelitis</td>
<td>Inactivated (attenuated also available)</td>
<td>Subcutaneous or intramuscular (attenuated: oral)</td>
</tr>
<tr>
<td>Influenza</td>
<td>Influenzaviruses</td>
<td>Flu</td>
<td>Inactivated subunit</td>
<td>Intramuscular or oral</td>
</tr>
<tr>
<td>Measles/ mumps/ rubella (MMR)</td>
<td>Measles virus</td>
<td>Measles</td>
<td>Attenuated</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mumps virus</td>
<td>Attenuated</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rubella virus</td>
<td>Attenuated</td>
<td></td>
</tr>
<tr>
<td>Varicella-zoster</td>
<td>Chickenpox virus</td>
<td>Chickenpox, shingles</td>
<td>Attenuated</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>Hepatitis A virus</td>
<td>Hepatitis A</td>
<td>Inactivated whole</td>
<td>Intramuscular</td>
</tr>
<tr>
<td><strong>Available But Not Recommended for General Population in the U.S.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthrax</td>
<td>Bacillus anthracis</td>
<td>Anthrax</td>
<td>Inactivated whole</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>BCG (bacillus of Calmette and Guérin)</td>
<td>Mycobacterium tuberculosis, M. leprae</td>
<td>Tuberculosis, leprosy</td>
<td>Attenuated</td>
<td>Intradermal</td>
</tr>
<tr>
<td>Japanese encephalitis vaccine</td>
<td>Japanese encephalitis virus</td>
<td>Encephalitis</td>
<td>Inactive</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>Rabies</td>
<td>Rabies virus</td>
<td>Rabies</td>
<td>Inactivated whole</td>
<td>Intramuscular or intradermal</td>
</tr>
<tr>
<td>Typhoid fever vaccine</td>
<td>Salmonella enterica</td>
<td>Typhoid fever</td>
<td>Attenuated (inactive also available)</td>
<td>Oral (inactive: subcutaneous or intramuscular)</td>
</tr>
<tr>
<td>Vaccinia (cowpox)</td>
<td>Smallpox virus, monkeypox virus</td>
<td>Smallpox, monkeypox</td>
<td>Attenuated</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>Yellow fever</td>
<td>Yellow fever virus</td>
<td>Yellow fever</td>
<td>Attenuated</td>
<td>Subcutaneous</td>
</tr>
</tbody>
</table>

Antisera have the following limitations:

- Repeated injections of horse-derived antisera can trigger a serious allergic response called serum sickness, in which the recipient mounts an immune response against horse antigens found in the antisera.
- Viral pathogens may contaminate antisera.
- The antibodies of horse-derived antisera are degraded relatively quickly by the recipient. The half-life of such antibodies is about 3 weeks; that is, half the molecules are degraded every 3 weeks.

Scientists have overcome the limitations of antisera by developing hybridomas (hi-brid-ó’ máz), which are tumor cells created...
Mouse is injected with antigen.

Hybridomas are formed by mixing and fusing plasma cells and myeloma cells; they are long lived and produce antibodies.

Hybridomas are placed individually in small wells and their antibodies are tested for reactivity against the antigen.

A hybridoma that makes antibodies that react with the antigen is cloned.

**Figure 17.4** The production of hybridomas. After a laboratory animal is injected with the antigen of interest, plasma cells are removed from the animal and isolated. When these plasma cells are fused with cultured cancer cells called myelomas, the results are hybridomas. Once the hybridomas are cultured individually and the hybridoma that produces antibodies against the antigen of interest is identified, it is cloned to produce a large number of hybridomas, all of which secrete identical antibodies called monoclonal antibodies.

Active immunization and passive immunotherapy are used in different circumstances because they provide protection with different characteristics (Figure 17.5). As just noted, passive immunotherapy with preformed antibodies is used whenever immediate protection is required. However, because preformed antibodies are removed rapidly from the blood and no memory B cells are produced, protection is temporary, and the recipient becomes susceptible again. Active immunization provides long-term protection that is capable of restimulation. Thus, when initiated before any exposure to *C. tetani* has occurred, active immunization using a tetanus toxoid develops long-lasting protection that is readily available upon exposure to the toxin.

**Antibody-Antigen Immune Testing**

**Learning Objectives**

- Distinguish between direct and indirect immune testing using antibody-antigen interactions.
- Define serology.
- In general terms, compare and contract precipitation, agglutination, neutralization, complement fixation, and labeled antibody tests.

There are two basic categories of immune testing using antibody-antigen interactions. For **direct testing**, the investigator is looking for the presence of an antigen, often a pathogen, in a specimen, which is typically taken from a site of infection. **Indirect testing** involves checking the blood or serum for antibodies that have formed against a particular antigen. Thus, in each category the
**Precipitation Tests**

**Learning Objectives**

✓ Describe the general principles of precipitation testing.
✓ Describe the technique of immunodiffusion.
✓ Discuss the production of anti-antibodies for immune testing.

---

The study and diagnostic use of antigen-antibody interactions in blood serum is called **serology** (sē-rol'-ö-jē). Researchers have developed a wide variety of serologic tests to visualize antibody-antigen interactions, ranging from simple, automated processes to complex tests requiring skilled technicians. Physicians and medical laboratory technologists choose particular tests based on the suspected diagnosis, the cost to perform the test, and the speed with which a result can be obtained. In the following subsections we will consider several types of serologic tests: precipitation tests, agglutination tests, neutralization tests, and several tagged antibody tests. Some of these procedures are presented for historical reasons—more accurate and faster modern tests have replaced them.

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**Precipitation Tests**

The reasons behind this pattern of precipitation reactions are simple. Complex antigens are generally multivalent—each possesses many epitopes—and antibodies have pairs of active sites and therefore can simultaneously cross-link the same epitope on two antigen molecules. When there is excess antibody, each antigen molecule is covered with many antibody molecules, preventing extensive cross-linkage and thus precipitation (Figure 17.6b). Such soluble immune complexes can activate complement in the kidneys, leading to inflammatory damage to blood vessels in the kidneys.

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**BENEFICIAL MICROBES**

**SMALLPOX: TO VACCINATE OR NOT TO VACCINATE?**

Dr. Edward Jenner developed an early use for a beneficial microbe in medicine. Medical personnel in the United States followed Jenner’s example by regularly administering cowpox virus—as the smallpox vaccine—to the general public until 1971, at which time the risk of contracting smallpox was deemed too low to justify required vaccinations. Indeed, in 1980 the World Health Assembly declared smallpox successfully eradicated from the natural world. However, recent concerns about the potential use of smallpox virus as an agent of bioterrorism has sparked debate about whether or not citizens should once again be vaccinated against it.

Although safe and effective for most healthy adults, for others the attenuated cowpox virus can result in serious side effects—even death. Individuals with compromised immune systems (such as AIDS patients or cancer patients undergoing chemotherapy) are considered to be at particularly high risk for developing adverse reactions. Pregnant women, infants, and individuals with a history of the skin condition eczema are also considered poor candidates for the vaccine. Though rare, adverse reactions to the vaccine may also develop in certain otherwise healthy individuals. The more serious side effects include vaccinia necrosis (characterized by progressive cell death in the area of vaccination) and encephalitis (inflammation of the brain). Approximately 1 in every million individuals receiving cowpox virus as a vaccine for the first time develops a fatal reaction to it.

Is the risk of a bioterrorist smallpox attack great enough to warrant the exposure to the known risks of administering smallpox vaccine to the general population? If you were a public health official, what would you decide?

---

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Is the risk of a bioterrorist smallpox attack great enough to warrant the exposure to the known risks of administering smallpox vaccine to the general population? If you were a public health official, what would you decide?

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**Vaccinia necrosum.**
When the reactants are in optimal proportions, the ratio of antigen to antibody is such that cross-linking and lattice formation are extensive. As this lattice grows, it precipitates.

In mixtures in which antigen is in excess, each antigen molecule is bound to only two antibody molecules. There is no cross-linkage; because these complexes are small and soluble, no precipitation occurs. In the body, phagocytic cells do not easily remove these small immune complexes. As a result, immune complexes may be deposited in the joints and in the tiny blood vessels of the kidneys, where they trigger allergic reactions, as discussed in Chapter 18.

Because precipitation requires the mixing of antigen and antibody in optimal proportions, it is not possible to perform a precipitation test by combining just any two solutions containing these reagents. To ensure that the optimal concentrations of antibody and antigen come together, scientists can use a technique involving movement of the molecules through an agar gel: immunodiffusion.

**Immunodiffusion**

In the precipitation technique called immunodiffusion (immú-nô-di-fu’zhûn), also known as double immunodiffusion or an Ouchterlony (ok’ter-lô-né) test, a researcher cuts cylindrical holes called wells in an agar plate. One well is filled with a solution of antigen and the other with a solution of antibodies against the antigen. The antigen and antibody molecules diffuse in all directions out of the wells and into the surrounding agar, and where they meet in optimal proportions, a line of precipitation appears (Figure 17.7a). If the solutions contain many different antigens and antibodies, each complementary pair of reactants reaches optimal proportions at different positions, and numerous lines of precipitation are produced—one for each interacting antigen-antibody pair (Figure 17.7b). Such an immunodiffusion test has
Increasing amounts of antigen placed in wells

Diffusion of antigens into agar

Agar containing antibodies to the specific antigen

Ring of precipitation

Diameter of ring is measured and compared to wells 1–5

(a)

(b)

Figure 17.8
Radial immunodiffusion, a type of precipitation reaction.
(a) Antigen placed into wells in increasing, known concentrations diffuses into agar containing a known concentration of antibody. The diameter of each ring of precipitation formed is proportional to the concentration of antigen in the well. (b) Plotting ring diameter versus known antigen concentration produces a standard curve; subsequently, the concentration of antigen in a solution can be determined by comparing the ring diameter produced by radial diffusion to the standard curve.

What is the antigen concentration of sample Y?

Based on the graph in (b), the concentration in sample Y is about 900 mg/100 ml.

been used to indicate exposure to complex mixtures of antigens from fungal pathogens. Only exposed patients have serum antibodies—and show precipitation—against the fungal antigens.

Another useful variation on precipitation is called radial immunodiffusion (Figure 17.8a). In this technique, an antigen in solution diffuses from a well into agar that contains a known concentration of specific antibodies. The diameter of the ring of precipitate that forms around the antigen well is directly proportional to the concentration of antigen in the well. By using a series of wells containing increasing but known concentrations of antigen, researchers construct a standard curve that relates ring diameter to antigen concentration (Figure 17.8b). Subsequently, the amount of antigen in a solution can be accurately assayed by comparing the ring diameter produced in other radial diffusion tests to the standard line. For example, investigators use radial immunodiffusion to measure the level of complement protein 3 (C3), which is often low in patients with lupus.

Scientists can also use radial immunodiffusion to measure the concentrations of specific antibodies or immunoglobulins in a person’s serum. To do this, they take advantage of the fact that, because antibodies are complex proteins, they are antigenic when injected into an individual of another species. Thus, purified human antibodies injected into a rabbit stimulate the rabbit to produce rabbit antibodies against the human antibodies. Such antibodies directed against other antibodies are called anti–antibodies. So, in a radial immunodiffusion test to measure the concentration of a specific human antibody, the “antigen” in the test is the human immunoglobulin, and the antibody in the test is rabbit anti–human antibody.

Agglutination Tests

Learning Objectives
✓ Contrast agglutination and precipitation tests.
✓ Describe how agglutination is used in immunological testing, including titration.

Not all antigens are soluble proteins that can be precipitated by antibody. Because of their multiple antigen-binding sites, antibodies can also cross-link particles, such as whole bacteria or antigen-coated latex beads, causing agglutination (ə-glo̅o-ti-nā′shūn) (clumping). The difference between agglutination and
Anti-A antibody added

Blood sample

Anti-B antibody added

A

B

Negative result: no agglutination of blood cells

Positive result: agglutination of blood cells

Figure 17.9 The use of hemagglutination to determine blood types in humans. (a) Antibodies with active sites that bind to either of two red blood cell surface antigens (antigen A or antigen B) are added to portions of a given blood sample. Where the antibodies react with the surface antigens, the blood cells can be seen to agglutinate or clump together. (b) Photo of actual test. What is the blood type of the person whose blood was used in this hemagglutination reaction?

precipitation is that agglutination involves the clumping of insoluble particles, whereas precipitation involves the aggregation of soluble molecules. Agglutination reactions are easy to see and interpret with the unaided eye. IgM antibodies, which have 10 active sites, are more efficient than IgG antibodies (with only 2 active sites) at causing agglutination.

When the particles agglutinated are red blood cells, the reaction is called hemagglutination (hë-mä-gloo’-ti-nä’shün). One use of hemagglutination is to determine blood type in humans. Blood is considered type A if the red blood cells possess surface antigens called A antigen, type B if they possess B antigens, type AB if they possess both antigens, and type O if they have neither antigen. In a hemagglutination reaction to determine blood type (Figure 17.9), two portions of a given blood sample are placed on a slide. Anti-A antibodies are added to one portion, and anti-B antibodies to the other; the antibodies agglutinate those blood cells that possess complementary antigens.

Another use of agglutination is in a type of test that determines the concentration of antibodies in a clinical sample. Although the simple detection of antibodies is sufficient for many purposes, it is often more desirable to measure the amount of antibodies in serum. By doing so, clinicians can determine whether a patient’s antibody levels are rising, as occurs in response to the presence of active infectious disease, or falling, as occurs during the successful conclusion of a fight against an infection. One way of measuring antibody levels in blood sera is by titration (tı-tra’shün). In titration, the serum being tested undergoes a regular series of dilutions, and each dilution is then tested for agglutinating activity (Figure 17.10). Eventually, the antibodies in the serum become so dilute that they can no longer cause agglutination. The highest dilution of serum giving a positive reaction is the titer. Thus, a serum that must be greatly diluted before agglutination ceases (for example, has a titer of 1000) contains more antibodies than a serum that no longer agglutinates after minimal dilution (has a titer of 10).

**Neutralization Tests**

**Learning Objectives**

✓ Explain the purpose of neutralization tests.
✓ Contrast a viral hemagglutination inhibition test with a hemagglutination test.
Neutralization tests work because antibodies can neutralize the biological activity of many pathogens and their toxins. For example, combining antibodies against tetanus toxin with a sample of toxin renders the sample harmless to mice because the antibodies have reacted with and neutralized the toxin. Next we briefly consider two neutralization tests that, although not simple to perform, effectively reveal the biological activity of antibodies.

**Viral Neutralization**

One neutralization test is viral neutralization, which is based on the fact that many viruses introduced into appropriate cell cultures will invade and kill the cells, a phenomenon called a cytopathic effect (seen in plaque formation, see Figure 13.17). However, if the viruses are first mixed with specific antibodies against them, their ability to kill culture cells is neutralized. In a viral neutralization test, the lack of cytopathic effects when a mixture containing serum and a known pathogenic virus is introduced into a cell culture indicates the presence of antibodies against that virus in the serum. For example, if a mixture containing an individual’s serum and a sample of hantavirus produces no cytopathic effect in a culture of susceptible cells, then it can be concluded that the individual’s serum contains antibodies to hantavirus, and these antibodies neutralized the virus. Viral neutralization tests are sufficiently sensitive and specific to ascertain whether an individual has been exposed to a particular virus or viral strain.

**Viral Hemagglutination Inhibition Test**

Because not all viruses are cytopathic—they do not kill their host cell—a neutralization test cannot be used to identify all viruses. However, many viruses (including influenzaviruses) have surface proteins that naturally clump red blood cells. (This natural process, called viral hemagglutination, must not be confused with the hemagglutination test we discussed previously—viral hemagglutination is not an antibody-antigen reaction.) Antibodies against influenzavirus inhibit viral hemagglutination; therefore, if serum from an individual stops viral hemagglutination, we know that the individual’s serum contains antibodies to the influenzavirus. Such viral hemagglutination inhibition tests can be used to detect antibodies against influenza, measles, mumps, and other viruses that naturally agglutinate red blood cells.

**The Complement Fixation Test**

**Learning Objective**

✓ Briefly explain the phenomenon that is the basis for a complement fixation test.

As we discussed in Chapter 15, activation of the classical complement system by antibody leads to the generation of membrane attack complexes (MACs) that disrupt cytoplasmic membranes (see Figure 15.9). This phenomenon is the basis for the complement fixation (kom-plè-ment fik-sa’shùn) test, which is a complex assay used to detect the presence of specific antibodies in an individual’s serum. The test can detect the presence of small amounts of antibody—amounts too small to detect by agglutination—though complement fixation tests have been replaced by other serological methods such as ELISA (discussed shortly) or genetic analysis using polymerase chain reaction (PCR; see Figure 8.5).

**Labeled Antibody Tests**

**Learning Objectives**

✓ List three tests that use labeled antibodies to detect either antigen or antibodies.
✓ Compare and contrast the direct and indirect fluorescent antibody tests, and identify at least three uses for these tests.
✓ Compare and contrast the methods, purposes, and advantages of ELISA and the western blot tests.

A different form of serologic testing involves labeled (or tagged) antibody tests, so named because these tests use antibody molecules that are linked to some molecular “label” that enables them to be detected easily. Labeled antibody tests using radioactive or fluorescent labels can be used to detect either antigens or antibodies. In the following sections we will consider fluorescent antibody tests, ELISA, and the western blot test.

**Fluorescent Antibody Tests**

Fluorescent dyes are used as labels in several important serologic tests. One of these dyes is fluorescein (flor-es’e-in), which can be chemically linked to an antibody without affecting the antibody’s ability to bind antigen. When exposed to ultraviolet light (as in a fluorescent microscope), fluorescein glows bright green. Fluorescein-labeled antibodies are used in direct and indirect fluorescent antibody tests.

**Direct fluorescent antibody tests** identify the presence of antigen in a tissue. The test is straightforward: A scientist floods a tissue sample suspected of containing the antigen with labeled antibody, waits a short time to allow the antibody to bind to the antigen, washes the preparation to remove any unbound antibody, and examines it with a fluorescent microscope. If the suspected antigen is present, labeled antibody will adhere to it, and the scientist will see fluorescence. This is not a quantitative test—the amount of fluorescence observed is not directly related to the amount of antigen present.

Scientists use direct fluorescent antibody tests to identify small numbers of bacteria in patient tissues. This technique has been used to detect Mycobacterium tuberculosis in sputum and rabies viruses infecting a brain. In one use, medical laboratory technologists employ a direct fluorescent antibody test to detect the presence of yeast in a sample (Figure 17.11).

**Indirect fluorescent antibody tests** are used to detect the presence of specific antibodies in an individual’s serum via a two-step process (Figure 17.12a):

1. After an antigen of interest is fixed to a microscope slide, the individual’s serum is added for long enough to allow serum antibodies, if present, to bind to the antigen. The serum is then washed off, leaving the antibodies bound to the antigen (but not yet visible).
2. Antibodies against human antibodies (anti–human antibody antibodies; in this example, anti-IgG) labeled with a
fluorescent dye are added to the slide and bind to the antibodies already bound to the antigen. After washing to remove unbound anti-antibodies, the slide is examined with a fluorescent microscope.

The presence of fluorescence indicates the presence of the labeled anti-antibodies, which are bound to serum antibodies bound to the fixed antigen; thus fluorescence indicates that the individual has serum antibodies against the antigen of interest.

Indirect fluorescent antibody testing is used to detect antibodies against many viruses and some bacterial pathogens, including *Neisseria gonorrhoeae* (ni-se’re-à go-nor-re’-ē), the causative agent of gonorrhea (Figure 17.12b).

Scientists routinely identify and separate B and T lymphocytes by using specific monoclonal antibodies produced against each cell type. The researchers can attach differently colored fluorescent dyes to the antibodies, allowing them to differentiate between types of lymphocytes. Such identification tests can quantify the numbers and ratios of lymphocyte subsets, information critical in diagnosing and monitoring disease progression and effectiveness of treatment in patients with AIDS and other immunodeficiency diseases.

**ELISAs**

In another type of labeled antibody test, called an enzyme-linked immunosorbent assay (im’-uhn-sôr’bent as’sà; ELISA), the label is not a dye, but instead an enzyme that reacts with its substrate to produce a colored product that indicates a positive test. One form of ELISA is used to detect the presence and quantify the abundance of antibodies in serum. This test, which often takes place in commercially produced plates, has five steps (Figure 17.13):

1. Each of the wells in the plate is coated with antigen molecules in solution.
2. Excess antigen molecules are washed off, and another protein (such as gelatin) is added to the well to completely coat any of the surface not coated with antigen.
3. A sample of each of the sera being tested is added to a separate well. Whenever a serum sample contains antibodies against the antigen, they bind to the antigen affixed to the plate.
4. Anti-antibodies labeled with an enzyme are added to each well.
The enzyme’s substrate is added to each well. The enzyme and substrate are chosen because their reaction results in products that cause a visible color change. A positive reaction in a well, indicated by the development of color, can occur only if the labeled anti-antibody has bound to antibodies attached to the antigen of interest. The intensity of the color, which can be estimated visually or measured accurately using a spectrophotometer, is proportional to the amount of antibody present in the serum.

ELISA has become a test of choice for many diagnostic procedures, such as determination of HIV infection, because of its many advantages:

- Like other labeled antibody tests, ELISA can detect either antibody or antigen.
- ELISAs are sensitive, able to detect very small amounts of antibody (or antigen).
- Unlike some diffusion and fluorescent tests, ELISA can quantify amounts of antigen or antibody.
- ELISAs are easy to perform.
- ELISAs are relatively inexpensive.
- ELISAs can simultaneously test many samples quickly.
- ELISAs lend themselves to efficient automation and can be read easily, either by direct observation or by machine.
- Plates coated with antigen and gelatin can be stored for testing whenever they are needed.

A modification of the ELISA technique, called an *antibody sandwich ELISA*, is commonly used to detect antigen (*Figure 17.14*). In testing for the presence of HIV in blood serum, for example, the plates are first coated with antibody against HIV (instead of antigen). Then the sera from individuals being tested for HIV are added to the wells, and any HIV in the sera will bind to the antibody attached to the well. Finally, each well is flooded with enzyme-labeled antibodies specific to the antigen. The name “antibody sandwich ELISA” refers to the fact that the antigen being tested for is “sandwiched” between two antibody molecules. Such tests can also be used to quantify the amount of antigen in a given sample.

**CRITICAL THINKING**

A diagnostician used an ELISA to show that a newborn had antibodies against HIV in her blood. However, six months later the same test was negative. How can this be?
Western Blot Test

A technique for detecting antibodies against multiple antigens in a complex mixture is a **western blot test**. The name “western blot” is a play on words that refers to the similarity of this technique to a Southern blot test (see Figure 8.7), named for the man who developed it. Western blots are also called **immunoblots**.

Western blot tests are currently used to verify the presence of antibodies against HIV in the serum of individuals who are antibody-positive by ELISA. Compared to other tests, western blot tests can detect more types of antibodies and are less subject to misinterpretation. A western blot test has three steps (Figure 17.15a):

1. **Electrophoresis.** Antigens in a solution (in this example, HIV proteins) are placed into wells and separated by gel electrophoresis. Each of the proteins in the solution is resolved into a single band, producing invisible protein bands.
2. **Blotting.** The protein bands are transferred to an overlying nitrocellulose membrane by absorbing the solution into absorbent paper—a process called blotting. The nitrocellulose membrane is then cut into strips.
3. **ELISA.** Each nitrocellulose strip is incubated with a test solution—in this example, those from each of six individuals.
who are being tested for antibodies against HIV. After the strips are washed, an enzyme-labeled anti-antibody solution is added for a time; then the strips are washed again and exposed to the enzyme’s substrate. Color develops wherever antibodies against the HIV proteins in the test solutions have bound to their substrates, as shown in the positive control. In this example, the individual tested in strip 3 is positive for antibodies against HIV, whereas the other five individuals are negative for antibodies against HIV. Colored bands common to all patients are normal serum proteins.

Recent Developments in Antibody-Antigen Immune Testing

Learning Objectives

✔ Discuss the benefits of using immunofiltration assays rather than an ELISA.

✔ Contrast immunofiltration and immunochromatographic assays.

Recent years have seen the development of simple immunoassays that give clinicians useful results within minutes. The most commonly used of these are immunofiltration and immunochromatography assays. These tests are not quantitative but rapidly give a positive or negative result, making them very useful in arriving at a diagnosis.

Immunofiltration (imm’-u-nó-fil-trá-shún) assays are rapid ELISAs based on the use of antibodies bound to a membrane filter rather than to plates. Because of the large surface area of a membrane filter, reactions proceed faster and assay times are significantly reduced as compared to a traditional ELISA.

Immunochromatographic (imm’-u-nó-kro’-mat-ó-graf’ik) assays are still faster and easier to read immunoassays. In these systems, an antigen solution (such as diluted blood or sputum) flowing through a porous material encounters antibody labeled with either pink colloidal gold or blue colloidal selenium. Where antigen and antibody bind, colored immune complexes form in the fluid, which then flows through a region where the complexes encounter antibody against them, resulting in a clearly visible pink or blue line, depending on the label used. These assays are used for pregnancy testing, which tests for human chorionic growth hormone—a hormone produced only by an embryo or fetus—and for rapid identification of infectious agents such as HIV, Escherichia coli (esh-ë-rík’ë-à ko’lë) O157:H7, group A Streptococcus, respiratory syncytial virus (RSV), and influenzaviruses. In one adaptation, the antibodies are coated on membrane strips, which serve as dipsticks. At one end, anti-antibodies are fixed in a line so that they cannot move in the membrane. The lower portion of the membrane is coated with antibodies against the antigen in question. These antibodies are linked to a color indicator in the form of a colloidal metal and are free to move in the membrane by capillary action.

Figure 17.16 illustrates the procedure used to test for the presence of group A Streptococcus in the nasal secretion of a patient. A technician prepares a nasal swab from the patient so as to release Streptococcus antigens if they are present. She then dips the membrane into the solution. The membrane’s antibodies bind to streptococcal antigens, forming complexes. The complexes move up the membrane by capillary action until they reach the line of anti-antibodies, where they bind and must stop because the anti-antibodies are chemically bound to the strip. Previously the complexes were invisible because they were dilute; now they are concentrated at the line of anti-antibodies and become visible, indicating that the patient had group A Streptococcus in his nose. The procedure from antigen preparation to diagnosis takes less than 10 minutes.

Table 17.3 on p. 508 lists some antibody-antigen immune tests that can be used to diagnose selected bacterial and viral diseases.
Chapter Summary

Immunization (pp. 491–498)

1. The first vaccine was developed by Edward Jenner against smallpox. He called the technique vaccination. Immunization is a more general term referring to the use of vaccines against rabies, anthrax, measles, mumps, rubella, polio, and other diseases.

2. Individuals can be protected against many infections by either active immunization or passive immunotherapy. **ANIMATIONS:** Vaccines: Function

3. Active immunization involves giving antigen in the form of either attenuated vaccines, inactivated (killed) vaccines, toxoid vaccines, or recombinant gene vaccines. Antibody titer refers to the amount of antibody produced. **ANIMATIONS:** Vaccines: Types

4. Pathogens in attenuated vaccines are weakened so that they no longer cause disease, though they are still alive or active and can provide contact immunity in unimmunized individuals who associate with immunized people.

5. Inactivated vaccines are either whole agent or subunit vaccines and often contain adjuvants, which are chemicals added to increase their ability to stimulate active immunity.

6. Toxoid vaccines use modified toxins to stimulate antibody-mediated immunity.

7. A combination vaccine is composed of antigens from several pathogens so they can be administered to a patient at once.

8. Having a large proportion of immunized individuals (>75%) in a population interrupts disease transmission, providing protection to unimmunized individuals. Such protection is called herd immunity.

9. Passive immunotherapy (a type of passive immunization) involves administration of an antiserum containing preformed antibodies. Serum sickness results when the patient makes antibodies against the antiserum.

10. The fusion of myelomas (cancerous plasma cells) with plasma cells results in hybridomas, the source of monoclonal antibodies, which can be used in passive immunization.

Antibody-Antigen Immune Testing (pp. 498–508)

1. Serology is the study and use of immunological tests to diagnose and treat disease or identify antibodies or antigens. Direct testing involves using antibodies to find an antigen in a specimen. Indirect testing involves using an antigen to find antibodies in serum or blood.

2. The simplest of the serologic tests is a precipitation test, in which antigen and antibody meet in optimal proportions to form immune complexes, which are often insoluble. Often this test is performed in clear gels, where it is called immunodiffusion.

3. Agglutination tests involve the clumping of antigenic particles by antibodies. The amount of these antibodies, called the titer, is measured by diluting the serum in a process called titration.

4. Antibodies to viruses or toxins can be measured using a neutralization test, such as a viral neutralization test. Infection by viruses that naturally agglutinate red blood cells can be demonstrated using a viral hemagglutination inhibition test.

5. The complement fixation test is a complex assay used to determine the presence of specific antibodies.

6. Fluorescently labeled antibodies—those chemically linked to a fluorescent dye—can be used in a variety of direct and indirect fluorescent antibody tests. The presence of labeled antibodies is visible through a fluorescent microscope.

7. Enzyme-linked immunosorbent assays (ELISAs) are a family of simple tests that can be readily automated and read by machine. These tests are among the most common serologic tests used. A variation of the ELISA is the western blot test, which is used to detect antibodies against multiple antigens in a mixture.

8. Immunofiltration assays and immunochromatographic assays are modifications of ELISA tests that can give much more rapid diagnostic results.

**TABLE 17.3**

**Antibody-Antigen Immune Tests and Some of Their Uses**

<table>
<thead>
<tr>
<th>Test</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunodiffusion (precipitation)</td>
<td>Diagnosis of syphilis, pneumococcal pneumonia</td>
</tr>
<tr>
<td>Agglutination</td>
<td>Blood typing; pregnancy testing; diagnosis of salmonellosis, brucellosis, gonorrhea, rickettsial infection, mycoplasma infection, yeast infection, typhoid fever, meningitis caused by <em>Haemophilus</em></td>
</tr>
<tr>
<td>Viral neutralization</td>
<td>Diagnosis of infections by specific strains of viruses</td>
</tr>
<tr>
<td>Viral hemagglutination inhibition</td>
<td>Diagnosis of viral infections including influenza, measles, mumps, rubella, mononucleosis</td>
</tr>
<tr>
<td>Complement fixation</td>
<td>In the past, diagnosis of measles, influenza A, syphilis, rubella, rickettsial infections, scarlet fever, rheumatic fever, infections of respiratory syncytial virus and <em>Coxiella</em></td>
</tr>
<tr>
<td>Direct fluorescent antibody</td>
<td>Diagnosis of rabies, infections of group A <em>Streptococcus</em>, identification of lymphocyte subsets</td>
</tr>
<tr>
<td>Indirect fluorescent antibody</td>
<td>Diagnosis of syphilis, mononucleosis</td>
</tr>
<tr>
<td>ELISA</td>
<td>Pregnancy testing; presence of drugs in urine; diagnosis of hepatitis A, hepatitis B, rubella; initial diagnosis of HIV infection</td>
</tr>
<tr>
<td>Western blot</td>
<td>Verification of infection with HIV, diagnosis of Lyme disease</td>
</tr>
</tbody>
</table>
Questions for Review

Answers to the Questions for Review (except Short Answer questions) begin on page A-1.

Multiple Choice

1. In order to obtain immediate immunity against tetanus, a patient should receive
   a. an attenuated vaccine of Clostridium tetani.
   b. a modified live vaccine of C. tetani.
   c. tetanus toxoid.
   d. immunoglobulin against tetanus toxin (antitoxin).
   e. a subunit vaccine against C. tetani.

2. Which of the following vaccine types is commonly given with an adjuvant?
   a. an attenuated vaccine
   b. a modified live vaccine
   c. a chemically killed vaccine

3. Which of the following viruses was widely used in living vaccines?
   a. coronavirus
   b. vaccinia virus
   c. influenza virus

4. When antigen and antibodies combine, maximal precipitation occurs when
   a. antigen is in excess.
   b. antibody is in excess.
   c. antigen and antibody are at equivalent concentrations.
   d. antigen is added to the antibody.
   e. antibody is added to the antigen.

5. An anti-antibody is used when
   a. an antigen is not precipitating.
   b. an antibody is not agglutinating.
   c. an antibody does not activate complement.
   d. an antigen is insoluble.
   e. the antigen is an antibody.

6. The many different proteins in serum can be analyzed by
   a. an anti-antibody test.
   b. a complement fixation test.
   c. a precipitation test.

7. A direct fluorescent antibody test requires which of the following?
   a. heat-inactivated serum
   b. fluorescent serum
   c. immune complexes

8. An ELISA uses which of the following reagents?
   a. an enzyme-labeled anti-antibody
   b. a radioactive anti-antibody

9. A direct fluorescent antibody test can be used to detect the presence of
   a. hemagglutination.
   b. specific antigens.
   c. antibodies.

10. Which of the following is a good test to detect rabies virus in the brain of a dog?
    a. agglutination
    b. hemagglutination inhibition
    c. virus neutralization

11. Attenuation is
    a. the process of reducing virulence.
    b. a necessary step in vaccine manufacture.
    c. a form of variolation.
    d. similar to an adjuvant.

12. An antisera is
    a. an anti-antibody.
    b. an inactivated vaccine.
    c. formed of monoclonal antibodies.
    d. the liquid portion of blood used for immunization.

13. Monoclonal antibodies
    a. are produced by hybridomas.
    b. are secreted by clone cells.
    c. can be used for passive immunization.
    d. all of the above

14. The study of antibody-antigen interaction in the blood is
    a. attenuation.
    b. agglutination.
    c. precipitation.
    d. serology.

15. Anti-human antibody antibodies are
    a. found in immunocompromised individuals.
    b. used in direct fluorescent antibody tests.
    c. formed by animals reacting to human immunoglobulins.
    d. an alternative method in ELISA.

True/False

1. _____ Passive immunotherapy provides more prolonged immunity than active immunization.

2. _____ It is standard to attenuate killed virus vaccines.

3. _____ One single serologic test is inadequate for an accurate diagnosis of HIV infection.

4. _____ ELISA is very easily automated.

5. _____ ELISA has basically replaced the western blot test.

Matching

Match the characteristic in the first column with the therapy it most closely describes in the second column. Some choices may be used more than once.

| A. Attenuated viral vaccine | 1. Induces rapid onset of immunity |
| B. Adjuvant | 2. Induces mainly an antibody response |
| C. Subunit vaccine | 3. Induces good cell-mediated immunity |
| D. Immunoglobulin | 4. Increases antigenicity |
| E. Residual virulence | 5. Uses antigen fragments |
|   | 6. Uses attenuated microbes |
CHAPTER 17 Immunization and Immune Testing

Labeling
Identify the chemicals represented by this artist’s conception of an ELISA.

1. ____________
2. ____________
3. ____________
4. ____________
5. ____________
6. ____________

Short Answer
1. Compare and contrast the Chinese practice of variolation with Jenner’s vaccination procedure.
2. What are the advantages and disadvantages of attenuated vaccines?
3. Compare the advantages and disadvantages of passive immunotherapy and active immunization.
4. How does precipitation differ from agglutination?
5. Explain how a pregnancy test works at the molecular level.
6. Compare and contrast herd immunity and contact immunity.

Concept Mapping
Using the following terms, draw a concept map that describes vaccines. For a sample concept map, see p. 93.
Or, complete this concept map online by going to the Study Area at www.masteringmicrobiology.com.

Active immunity
- Antigenic fragments
- Attenuated whole agents
- Hepatitis B vaccine
- Immunizations

Inactivated whole agents
- Inactivated polio vaccine
- Killed vaccine
- Live vaccines
- Measles vaccine

Microorganisms
- Modified toxins
- Reduced virulence
- Subunits
- Tetanus toxoid

Toxoids
- Varicella vaccine

Critical Thinking
1. Is it ethical to approve the use of a vaccine that causes significant illness in 1% of patients, if it protects immunized survivors against a serious disease?
2. Which is worse: to use a diagnostic test for HIV that may falsely indicate that a patient is not infected (false negative), or to use one that sometimes falsely indicates that a patient is infected (false positive)? Defend your choice.
3. Discuss the importance of costs and technical skill in selecting a practical serologic test. Under what circumstances does automation become important?
4. What bodily fluids, in addition to blood serum, might be usable for immune testing?
5. Why might a serologic test give a false positive result?
6. Some researchers want to distinguish between B cells and T cells in a mixture of lymphocytes. How could they do this without killing the cells?
7. Describe three ways by which genetic recombinant techniques could be used to develop safer, more effective vaccines.
8. How does a toxoid vaccine differ from an attenuated vaccine?
9. Explain why many health organizations promote breast feeding of newborns. What risks are involved in such nursing?
10. Contrast a hemagglutination test with a viral hemagglutination inhibition test.
11. Sixty years ago parents would have done almost anything to get a protective vaccine against polio for their children. Now parents fear the vaccine, not the disease. Why?

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- Animations
- MP3 Tutor Sessions
- Concept Mapping Activities
- Flashcards
- Quizzes
and more to help you succeed.