

## Appendix A: Immunological Concepts

### Immunity

Immunology is the study of the immune system. The body protects itself from infection using physical and chemical barriers, antibodies that circulate in the blood, and immune cells that attack foreign substances and invading microorganisms. Some types of immune cells adapt to “remember” (recognize) specific invaders, in case of future attacks.

A person is born with certain immunological defenses against pathogens. This is called **innate immunity** and includes circulating macrophages and natural killer cells. These defenses do not change with exposure to pathogens and do not have much specificity for particular pathogens.

**Passive immunity** is the acquisition of antibodies from an external source, for example, antibodies passed from mother to infant, or certain postexposure vaccines such as that for rabies. Passive immunity lasts only a few weeks, and also does not change with multiple exposures.

**Acquired** or adaptive immunity is a specific response to specific foreign substances. Although individuals (except for those individuals who are immune-compromised) are born with the ability to respond to these invaders, the system must be activated by an initial contact with the invader. The initial contact, or immunization, begins a cascade of events that allows the body to mount a specific response on subsequent exposure to the invader, hence the term acquired immunity, as initial contact is necessary to acquire the immunity. Acquired immunity is split into two categories: **humoral immunity** involves production of antibodies that circulate in the bloodstream and lymph and bind specifically to foreign antigens, and **cell-mediated immunity** involves the production of T lymphocytes (T cells) that bind and destroy infected cells.

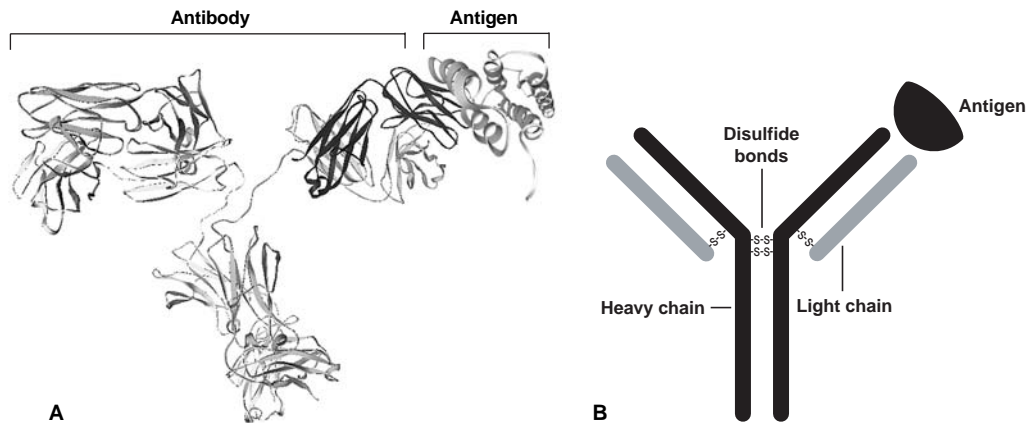
Acquired immunity is the basis for the series of vaccinations that we undergo as we grow up. In the 1790s, long before we had any understanding of the immune system, it was discovered that inoculation with pus from a cowpox lesion prevented infection with smallpox, a disease related to cowpox. The US Centers for Disease Control (CDC) currently recommends childhood vaccination against 12 diseases: measles, mumps, rubella (German measles), diphtheria, tetanus (lockjaw), pertussis (whooping cough), polio, *Haemophilus influenzae* type b (Hib disease), hepatitis B, varicella (chicken pox), hepatitis A, and pneumococcal disease. For travelers abroad, additional vaccinations are recommended (or required, in the case of the US military). The recommendations are based on the traveler's destination. For example, the CDC recommends that travelers to tropical South America be vaccinated against hepatitis A, hepatitis B, rabies (if the traveler will be exposed to animals), typhoid, and yellow fever, plus booster doses for tetanus, diphtheria, and measles.

### Components of the Acquired Immune Response

In an immune response, an invasion by something foreign to the body (an **antigen**) generates **antibody** production by B lymphocytes (B cell). Each B lymphocyte generates a unique antibody that recognizes a single shape on an antigen called an **epitope** and thus helps the **immune cells** (including B cells, T cells, and macrophages) to recognize and attack foreign invaders. Everyone (except those who are immune-compromised) has circulating antibodies and lymphocytes that collectively recognize a huge number of antigenic substances.

**Antigens** can be microorganisms (e.g., viruses and bacteria), microbial products (e.g., toxins produced by some bacteria, or protein components of the microbes), foreign proteins, DNA and RNA molecules, drugs, and other chemicals.

**Antibodies** are proteins also called immunoglobulins (Ig), that are produced by B cells and can remain attached to B cells or become free floating. There are five classes of immunoglobulins: IgG, IgM, IgA, IgE, and IgD. IgG is the most abundant (Parham) in the internal body fluids, comprising about 15% of total serum protein in adults, and each IgG molecule can bind two antigen molecules. IgM is also in serum and is responsible for the primary immune response. IgA is found in external secretions such as tears, saliva, milk, and mucosal secretions of the respiratory, genital, and intestinal tracts and is a first line of defense against invading microorganisms. IgA is also the only antibody passed from mother to infant. IgD may be involved in regulating the immune response, and IgE is a primary component in allergic reactions.



**A)** Structure of IgG bound to the HIV capsid protein p24 as determined by X-ray crystallography (Harris et al. 1998, Momany et al. 1996). These structures can be downloaded from the Protein Data Bank ([www.pdb.ubm.de](http://www.pdb.ubm.de), Berman et al. 2000) using the PDB identification codes 1IGY and 1AFV and manipulated using free online software such as Rasmol and Protein Explorer. **B)** A commonly used representation of an antibody bound to an antigen.

**Epitopes** are the specific parts of antigens that are recognized by antibodies. Each antibody recognizes a single epitope, thus multiple antibodies may recognize and bind to different epitopes on a single antigen. For example, an HIV virus particle (virion) has many potential epitopes on its surface that may be recognized by many different antibodies. One particular antibody may recognize the amino terminus of p24, an HIV capsid protein, while another may recognize the carboxy terminus of p24.

**Immune cells** are the soldiers of the acquired immune response. Macrophages serve two primary functions: 1) removing foreign cells and molecules from the blood, and 2) processing antigens and presenting them on their cell surfaces. Macrophages present antigenic epitopes on their cell surfaces to be recognized by T cells. The T cells draw more immune cells to the site of infection, causing inflammation. Both B cells and T cells are lymphocytes (white blood cells), and each recognizes a single specific epitope. T cells mature in the thymus, and B cells mature in the bone marrow. B cells produce antibodies; the number of different circulating antibodies has been estimated to be between  $10^6$  and  $10^{11}$ , so there is usually an antibody ready to deal with any antigen. The huge number and diversity of different antibodies are possible because B cells have the ability to rearrange their DNA to make different antibody genes. Like macrophages, B cells present antigenic

epitopes on their surface to attract T cells. T cells have two main functions: they stimulate the proliferation of B cells that have bound to an antigen, and they kill whole cells that are infected by a virus to prevent the virus infecting other cells.

### Why We Need an Immune System

Even bacteria have a rudimentary innate immune system; they make restriction enzymes that destroy foreign DNA from bacterial viruses (bacteriophages), and they protect their own DNA by labeling it as “self” through methylation. Our immune system is at work every day, protecting us from thousands of potential threats, but it is so efficient that we usually don’t notice it. Disease can result from infection, genetic defect, or environmental toxins.

**Infection** is an invasion by and multiplication of pathogenic (disease-causing) microorganisms. The infection can be 1) transmitted from person to person, like a cold or the flu, 2) transmitted from animals to people (called zoonosis), like rabies or psittacosis, or 3) contracted from the environment, like parasites contracted from water or soil.

The CDC and World Health Organization (WHO) state that **infectious diseases** are the leading cause of death worldwide. Organisms that can cause disease are called **pathogens** and include bacteria, viruses, fungi, infectious proteins called prions, and parasites.

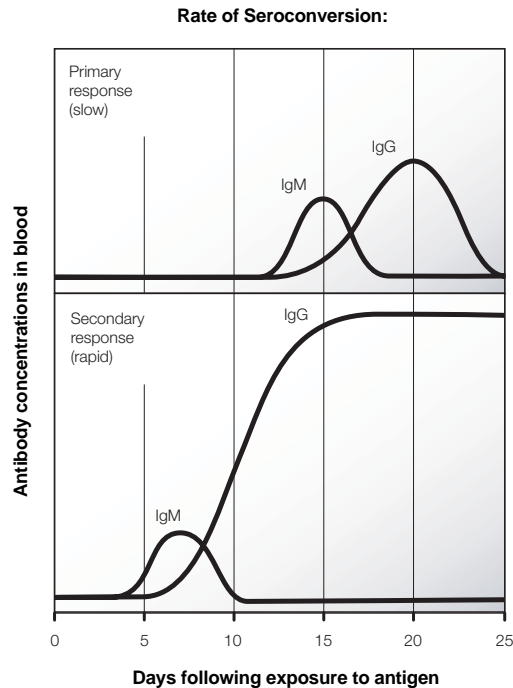
Infectious diseases spread in a variety of ways:

Pathogen Spread Through:	Examples
Exchange of body fluids	<ul style="list-style-type: none"> <li>HIV, SARS, Epstein-Barr virus (EBV), sexually transmitted diseases</li> </ul>
Food	<ul style="list-style-type: none"> <li>Foodborne agents like <i>E. coli</i> O157:H7, which causes diarrheal disease; prions, which cause Creutzfeldt-Jakob disease (mad cow disease in cattle); or nematodes, which cause trichinosis</li> </ul>
Water	<ul style="list-style-type: none"> <li>Waterborne agents like the bacteria that cause cholera or the protozoa that cause giardiasis</li> </ul>
Inhalation	<ul style="list-style-type: none"> <li>Microorganisms like the viruses that cause the flu or the bacteria that cause tuberculosis</li> </ul>
Absorption through the skin	<ul style="list-style-type: none"> <li>Nematodes like hookworms</li> </ul>
Vector transfer (vectors are organisms such as ticks or mosquitoes that carry pathogens from one host to another).	<ul style="list-style-type: none"> <li>Malaria, West Nile virus, dengue fever, and yellow fever (mosquito vector)</li> <li>Lyme disease and Rocky Mountain spotted fever (both tick vectors)</li> <li>Plague (flea vector)</li> <li>Some diseases, such as Ebola hemorrhagic fever, are presumed to have vectors, but the vectors have not yet been identified</li> </ul>

## Immune Response

When immunized with a foreign substance (either by vaccination or through natural exposure), an individual mounts an immune response, called the **primary response**. Within 1–2 weeks, there is a rise in antibody production directed against the antigen (termed seroconversion), predominated by the IgM class of antibodies. IgM production is usually followed by production of IgG, and after that antibody levels decrease.

The second time that the individual is exposed to the antigen, be it weeks or years after immunization, the immune response is larger and much more rapid. In the **secondary response**, IgM is produced in detectable amounts in a matter of days, followed by a large production of IgG. Other classes of immunoglobulin may also be produced. IgG is generated in much greater quantities, and persists in the blood for a much longer time than in the primary response. Antibody production may continue for months or even years.



## Problems With the Immune System

We depend on our immune system to protect us from disease, but when the immune system fails to function correctly, it can cause severe health problems. These problems fall in to three basic categories: hypersensitivity, immunodeficiency, and autoimmune diseases.

**Hypersensitive reactions** occur when the immune system overreacts to an antigen. The immune system functions are normal in a hypersensitive reaction, just exaggerated in scope, and this can result in illness or even death. There are four types of hypersensitive reactions: 1) anaphylactic reactions or immediate hypersensitivity, generally called allergies, such as food, dust mite, and pollen allergies (the antigen that causes the reaction is called an allergen); 2) cytotoxic reactions, such as transfusion reactions and Rh incompatibility reactions; 3) immune complex reactions, such as farmer's lung, a disease caused by inhaling mold spores; and 4) delayed-type hypersensitivity, such as contact sensitivity (e.g., poison ivy dermatitis and contact dermatitis after exposure to chemicals or environmental agents ranging from metallic nickel to cosmetics).

**Immunodeficiency** means that an individual is unable to mount an effective immune response, resulting in increased vulnerability to opportunistic infections. There are two types of immunodeficiency: 1) Primary immunodeficiency has a genetic basis. Severe combined immunodeficiency (SCID, "bubble boy" disease) is an example of primary immunodeficiency. Treatments for primary immunodeficiency may include gene therapy. 2) Secondary immunodeficiency has an external cause and is more common than primary immunodeficiency. Secondary immunodeficiency may be caused by an infection, as in the case of HIV/AIDS, by drug treatments, such as immunosuppressive drugs given after organ transplant, or by other health factors, such as poor nutrition, stress, or aging.

**Autoimmune disease** results from the immune system making a mistake and mounting an immune response against one's own body. Some examples of autoimmune disease include systemic lupus erythematosus (lupus, SLE), rheumatoid arthritis, multiple sclerosis (MS), insulin-dependent diabetes mellitus (IDDM), and celiac disease.

### **Detecting Infectious Diseases**

Infectious diseases are diagnosed by observing symptoms and performing laboratory tests. Diagnostic tests may look for the microorganism itself or some part of it (e.g., bacterial or viral antigens), microbial products (e.g., bacteria toxins), or reactions of the body to the disease agent. The latter may include testing for signs of an immune response to the disease agent (e.g., antibodies) or for indications of effects of the disease agent on the body (e.g., abnormal enzyme activity or protein levels). In the last decade, tests to detect microbial RNA and DNA have become common.

Laboratory tests cover a wide variety of methods, some of which have been in use for decades and others, like the tests for RNA and DNA from disease agents, which are very new. Depending on the test and putative diagnosis, laboratory tests may look for signs of disease in most body fluids, including blood, urine, stool samples, cerebrospinal fluid, and saliva. In the US, the Food and Drug Administration regulates laboratory tests.

The first tests for detecting and identifying microorganisms from clinical samples used antisera directed against specific microbes. The antibodies were labeled with a fluorescent tag, and the microorganisms could be detected with microscopy when the antibodies bound to them. Other early diagnostic tests include: 1) culture methods, in which microorganisms from clinical samples are grown on different culture media and their growth and appearance observed (frequently takes weeks to get results); 2) identification of microbe-specific antibodies in serum by immunoassays such as ELISA; and 3) agar diffusion assays, in which antisera and antigens are placed in holes in agar plates. Both diffuse into the agar, and where antibodies encounter antigens for which they are specific, they bind. Upon antibody-antigen binding, a visible precipitation band forms. Many of these tests are still in use.

Current diagnostic tests include:

Test Type	Description	Examples
Immunofluorescence assay (IFA)	Specific microorganisms detected with fluorescently labeled antibodies	<ul style="list-style-type: none"> <li>• <i>E. coli</i> O157:H7</li> <li>• Identifying respiratory viruses</li> </ul>
Agglutination	Visible precipitates appear when antibodies and specific antigens come in contact	<ul style="list-style-type: none"> <li>• Gram-positive bacteria, such as <i>Staphylococcus aureus</i> (e.g., Staph A)</li> <li>• Fungi, such as <i>Cryptococcus neoformans</i> and <i>Candida</i> species</li> </ul>
Immunochromatography tests	Card or dipstick-based immunoassays	<ul style="list-style-type: none"> <li>• <i>E. coli</i> O 157:H7</li> <li>• <i>Legionella</i></li> <li>• <i>Mycoplasma pneumoniae</i></li> </ul>
Microplate tests	ELISA or RIA (radioimmunoassays) used to detect microbial antigens, microbial products, and antibodies against the microorganisms and their products. RIA uses radioactive labels to replace the enzymes used in ELISA	<ul style="list-style-type: none"> <li>• <i>E. coli</i> O157:H7</li> <li>• <i>Legionella</i></li> <li>• Influenza viruses</li> <li>• HIV antigen</li> <li>• HIV antibodies</li> <li>• <i>Giardia</i></li> </ul>
Molecular methods	Detection of microbial RNA or DNA; also used to detect microbial drug resistance (AST); may use PCR	<ul style="list-style-type: none"> <li>• <i>Mycobacterium tuberculosis</i> (TE)</li> <li>• HIV</li> <li>• <i>Chlamydia trachomatis</i></li> <li>• Cytomegalovirus (CMV)</li> <li>• AST (antimicrobial susceptibility testing)</li> </ul>
Microscopy	Visual identification based on staining with specific reagents or on physical characteristics	<ul style="list-style-type: none"> <li>• Electron microscopy of Ebola virus</li> <li>• Light microscopy of parasites such as protozoa, helminthes, etc.</li> </ul>

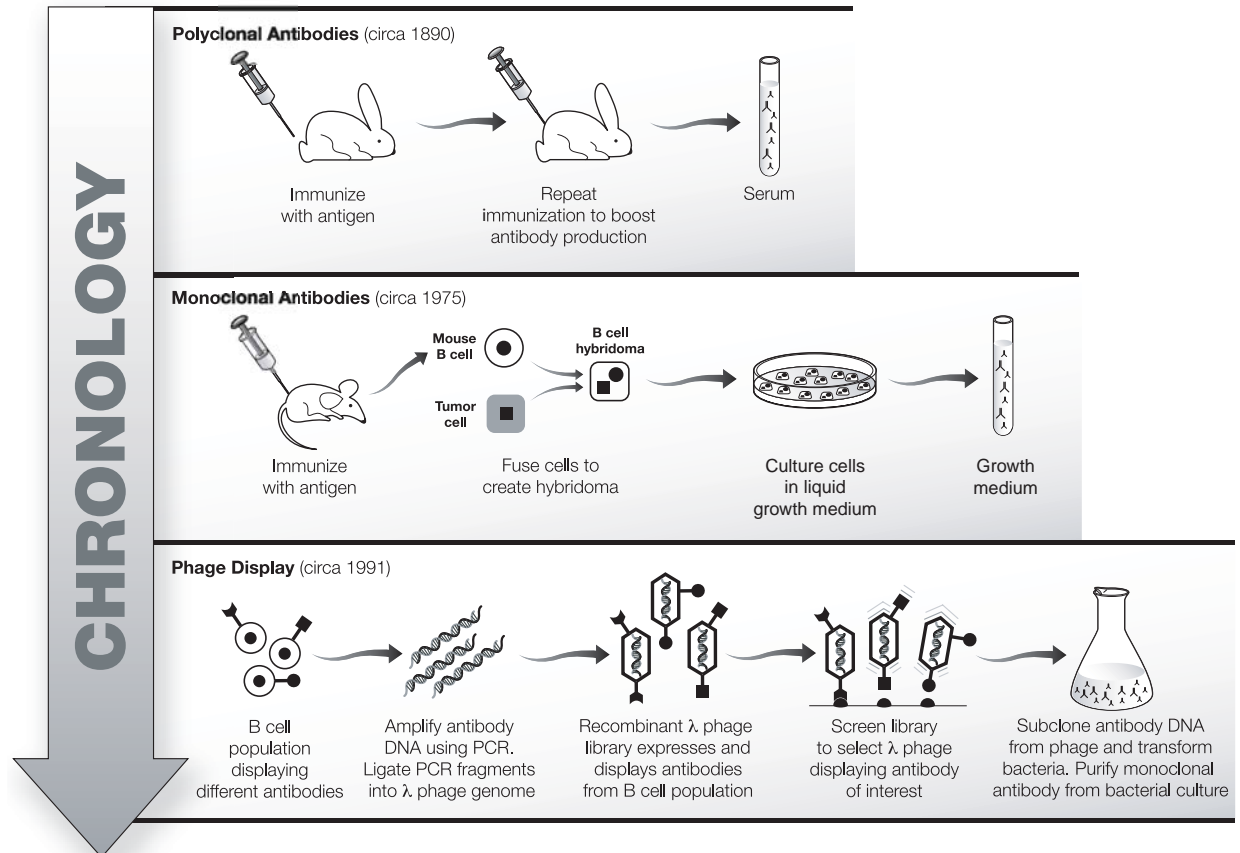
## Boosting the Immune System With Vaccination

Doctors use the immune response to give us resistance to infectious diseases before we are exposed to them. Through vaccination, we are exposed to non-harmful forms of the pathogen that invoke an immune response. We also frequently need booster shots to invoke the secondary response to maintain the antibody levels in our blood. Vaccines used in immunization may be of several types:

- 1) **Live attenuated vaccines** are weakened (attenuated) microbes, that are nonpathogenic. Using current technology, deletion or inactivation of microbial genes weakens the pathogens so they can be used in vaccines; previously, less pathogenic strains were selected from natural populations. Examples of live vaccines include those against polio (Sabin type), measles, mumps, and smallpox.
- 2) **Killed or inactivated vaccines** are made of microbes killed by heat or chemicals. Killed vaccines are much safer than live vaccines, particularly for individuals with compromised immune systems, but they do not usually provoke as strong an immune response as do live vaccines. Examples of killed vaccines include those against rabies, cholera, polio (Salk type), and influenza.
- 3) **Subunit vaccines** are made from pieces of microbes. They consist of one or more antigens from either the disease agent or a microbial product, and they may be derived from the organisms or engineered using molecular biology. Examples of subunit vaccines include those against hepatitis B, anthrax, and tetanus.
- 4) **DNA vaccines** are a recent approach to vaccine development. DNA that codes for microbial antigens is cloned into a vector, and the naked DNA is injected into the patient. The DNA is taken up by cells, transcribed, and translated, and the resulting antigenic protein elicits an immune response. No DNA vaccines are yet available, but some are in clinical trials.
- 5) **Antibody vaccines** are another innovation in vaccine development. The ability to construct human monoclonal antibodies using recombinant DNA technology means that antibodies prepared against specific antigens may be used safely in humans. For example, a human monoclonal antibody against an antigen involved in anthrax infection may soon be in clinical trials.
- 6) **Postexposure vaccines (immunotherapy)** are used to treat a disease. Some immunotherapies have been used for years (e.g., administering immune serum globulin after exposure to hepatitis and administering equine antivenin for snakebite), but there are not many other current vaccine-based immunotherapies. Probably the best known is postexposure rabies vaccination, consisting of 5 doses of rabies vaccine over 30 days. If the vaccine regimen is begun promptly after exposure, it is 100% effective in preventing disease. Smallpox vaccination also provides protection even when administered 2–3 days postexposure. If the smallpox vaccine is administered as late as 5 days after exposure, it may prevent smallpox from being fatal, although it will not prevent the disease.

## Tapping Nature's Toolkit: Manufacturing Antibodies

Antibodies used in research can be manufactured in the laboratory, both in vivo and in vitro. In vivo techniques have been in use for over 100 years. There are two types of traditionally produced antibodies: **polyclonal antibodies** and, in the last 30 years, **monoclonal antibodies**. Currently, antibody production is being revolutionized by recombinant DNA technology and, while most antibodies are still produced by traditional methods using animals or animal cells, techniques for making antibodies using recombinant DNA technology are becoming more common.



Timeline of antibody production technology.

### Polyclonal Antibodies

Polyclonal antibodies are generated by immunizing an animal (usually a rabbit, goat, or sheep) and obtaining serum. For example, purified HIV gp120 protein can be injected into a goat, which will then generate antibodies directed against the many epitopes of gp120. (Remember that the goat will produce many different antibodies to the multiple epitopes of an antigen.) Blood containing the antibodies is drawn from the goat and the cells of the blood are removed, leaving the serum. The product is **antiserum** towards gp120, and the antiserum can be used directly or the antibodies can be purified from it. The antibodies are called polyclonal because the antibodies are from many (*poly*) B cell clones (*clonal*) in the goat's blood. Polyclonal antiserum has the advantage of being simple and inexpensive to produce, but the disadvantage is that no two batches, even made in the same animal, will be exactly the same.



### **Monoclonal Antibodies**

For many antibody applications such as diagnostic tests, polyclonal antibodies are too variable. In these cases, one antibody type from a single B cell clone is preferable. B cell clones producing single antibodies can be isolated from the spleens of immunized mice, but these cells die after a few weeks in the laboratory, limiting production of the large amounts of antibody generally needed for research and commercial applications. However, B cells can be made to live (and produce antibodies) indefinitely if they are fused with tumor-like immortal cells. The fusion generates hybrid cells (a hybridoma cell line), which can be cultured indefinitely; the monoclonal antibodies generated by the hybrid cells can be collected and purified from the growth medium with almost no batch-to-batch variability.

### **Genetically Engineering Antibodies**

The ability of antibodies to act like magic bullets and home in on their targets makes them ideal candidates for medical therapies. For example, an antibody that recognizes a tumor antigen can be attached to a chemotherapy drug or radioactive molecule and be used to deliver the drug specifically to targeted tumor cells, sparing the patient many of the side effects of conventional chemotherapy or radiation treatment. However, traditional antibodies made in animals are seen by the human immune system as foreign and elicit an immune response that results in their destruction. Recombinant DNA technology can be used to produce antibodies that look human to the human immune system and so can be used as therapeutic agents in people. (For example, Herceptin is a “humanized” antibody used to treat breast cancer.) Using genetic engineering to manufacture antibodies also obviates the sacrifice of laboratory animals. Two of the methods used to engineer antibodies are described below.

### **Hybridoma Immortalization**

Recombinant DNA technology allows the antigen recognition site from a known mouse monoclonal antibody to be camouflaged within a human antibody by combining part of the mouse gene with the human antibody gene. Bacteria transformed with this DNA are capable of producing humanized monoclonal antibodies indefinitely, with the added bonus that culturing bacteria requires much less time and expense than the culture of a mouse hybridoma cell line.

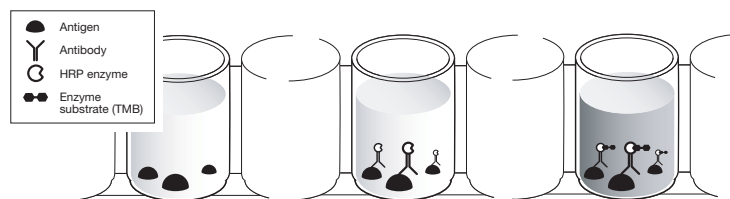
### **Phage Display**

Novel antibodies to antigens are being generated using modern biotechnology. Libraries of billions of potentially useful antibodies are being created by inserting shuffled antibody genes from billions of human B cells into the genomes of bacteriophage lambda (bacteriophages, or phages, are viruses that infect bacteria; lambda phage is a specific species of phage), so that the lambda phages display the binding sites from human antibodies on their surfaces. This **phage library** is screened to find a phage that binds to a specific antigen. The phage can then be used directly as an antibody would be used. Alternatively, the DNA from the selected phage can be cloned into a human antibody gene and transformed into bacteria. Large amounts of the antibody can then be produced for therapeutic use. Phage display is on the cutting edge of immunotherapy.

## Labeling and Detecting Antibodies

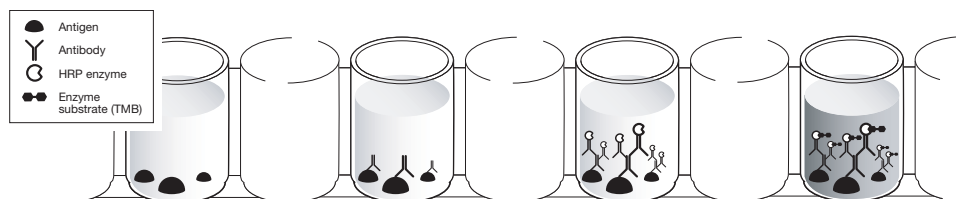
Antibodies are used in diagnosis and research as labeling tools. As labels they have to be made visible, so antibodies are covalently linked (or conjugated) to chemical labels that emit detectable signals. Detection systems can be low-tech or high-tech, and the detection system determines the type of label used. For example, a fluorescently labeled antibody allows you to localize an antigen in a cell using a high-tech fluorescent microscope. Antibodies are also linked to enzymes that oxidize a chromogenic (color-producing) substrate, producing visible color only where the enzyme-linked antibody has bound. Enzyme-linked antibodies are commonly used in western blots, microscopy, and ELISA.

Antibody targets or antigens can be detected directly by labeling the antibody specific for the antigen and looking for signal.



### Direct detection of antibodies.

However, labeling every type of antibody scientists might wish to use is time-consuming and costly. Thus, a more common method to visualize antigens is called indirect detection. This technique relies on the use of polyclonal secondary antibodies. Secondary antibodies recognize primary antibodies. The primary antibody binds specifically to the antigen, and the secondary antibody binds specifically to the primary antibody. The indirect method means that only one type of enzyme-linked secondary antibody is needed to visualize all antibodies produced in one type of animal (e.g., in rabbits), reducing time and cost. Indirect detection adds a bonus, since the primary antibody is effectively an antigen to the secondary antibody. The primary antibody has many different epitopes and so is bound by multiple secondary antibodies. Thus, more labels accumulate around the antigen, amplifying the signal.



### Indirect detection of antibodies.

Secondary antibodies are produced by injecting the antibodies of one animal into a different species of animal. For example, if the primary antibody is a mouse monoclonal antibody, secondary antibodies are generated by immunizing a goat with any mouse antibody. Goat polyclonal anti-mouse IgG is purified from the goat serum and linked to an enzyme for detection. Secondary antibodies are commercially available, either unlabeled or with a wide variety of fluorescent or enzymatic labels for many applications.

### **Putting Antibodies to Use**

Antibodies have been used for decades as research tools, but in recent years the expansion of technology to produce antibodies has yielded a myriad of new applications that take advantage of the specificity of antibody binding. The basis of all immunoassays is the specific binding of an antibody to its antigen, and there are many ways that binding can be utilized. Here are some of those uses:

**Immunostaining** localizes antigens in organelles, cells, tissues, or whole organisms, and can also be used to distinguish one cell type from another. For example, pathologists can identify cancer cells using immunostaining. Cancer cells frequently look identical to normal cells under the microscope, but when they are immunostained, variations in the amount and kinds of cell surface proteins (antigens) are revealed. Studying this information helps diagnose cancer, and it can help in our understanding of how cancer cells cause harm.

Immunostaining tissues or organisms can tell us in what cell types a protein is normally found, which can help us understand the protein's function. For instance, immunostaining of plant seedlings at different stages of maturation allows us to follow how a protein's abundance and localization change as the plant grows. Antibodies for immunostaining are labeled with either fluorescent molecules or enzymes that produce colored signals upon addition of a substrate.

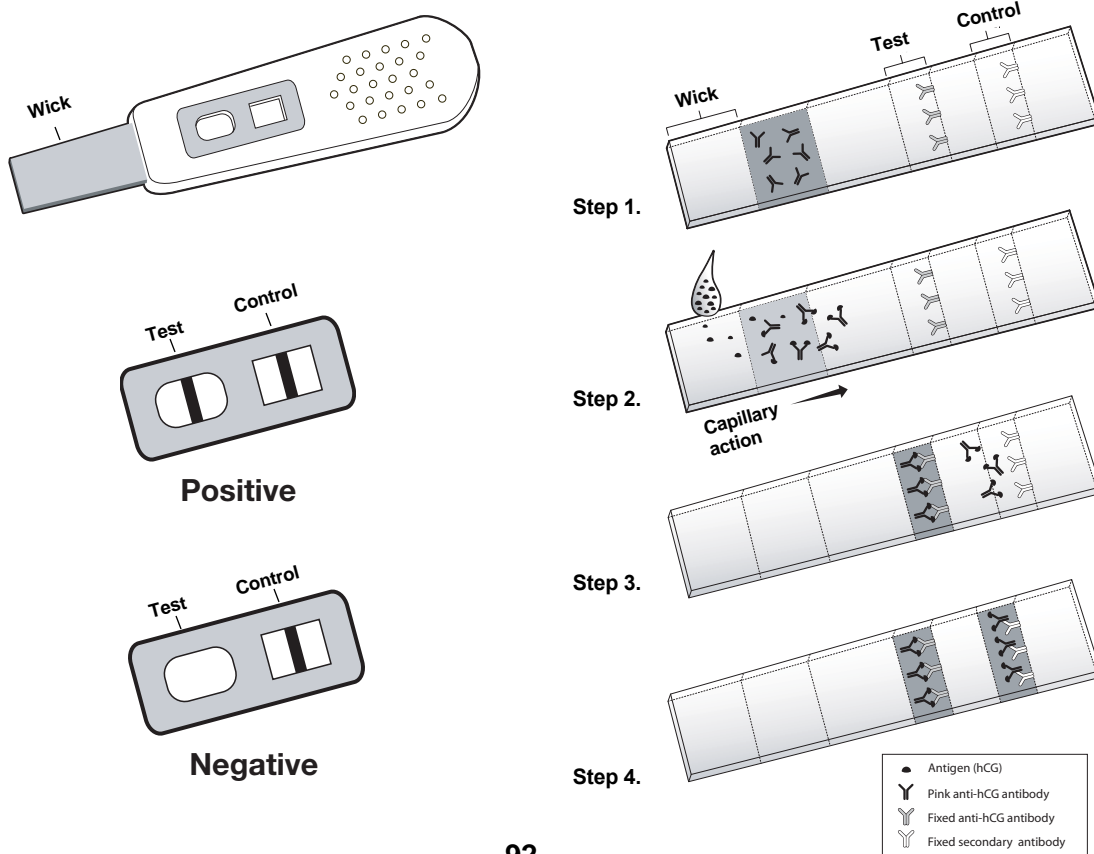
A special application of immunostaining is fluorescence-activated cell sorting (FACS), in which a population of cells is stained with a fluorescently labeled antibody and then physically separated into labeled and unlabeled cells. The cell sorter uses lasers to detect the fluorescent labels and an electrostatic charge to sort the cells in solution. Cell sorters can separate as many as 30,000 cells per second!

**Immunoblotting or western blotting** tells us about a protein's size and relative abundance in a given sample. In western blotting, an antibody picks out a specific protein from a complex sample (usually lysed cells or tissue) that has been separated by size using SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins separated in SDS-PAGE gels are transferred (electroblotted) from the gel to the surface of a nylon or nitrocellulose membrane using an electrical current. The membrane is probed with a primary antibody that is specific for the protein of interest, and then an enzyme-linked secondary antibody is used to visualize the protein. The enzyme oxidizes a colorimetric substrate, producing a colored band on the membrane. Alternatively, the oxidized substrate may emit light (chemiluminescent substrate) that is detected as a band on photographic film. The size of the protein is determined by comparing the position of the band to the position of known protein standards that are run alongside it on the SDS-PAGE gel. The abundance of the protein is determined by comparing band intensity to known amounts of protein standards run on the same gel.

A modification of immunoblotting is called **dot blotting**, in which a sample is spotted onto a membrane directly rather than being blotted from a gel. Dot blotting is used for rapid

screening of a large number of samples. This technique provides a rapid determination of whether a particular protein or antigen is present, as many samples may be spotted on a membrane and processed simultaneously, but dot blotting provides no information about the size of the protein.

There are many varieties of **dipstick tests**, including home pregnancy tests, tests for illegal drug use (e.g., marijuana, cocaine, and methamphetamines), and tests for infectious agents (e.g., HIV, plague, *E. coli* O157, and *Legionella*). These immunochromatography assays give positive or negative results in a matter of minutes and are called sandwich immunoassays because they rely on the use of multiple antibodies. One of the antibodies is labeled with a colored compound, such as colloidal gold, which produces a pink band on the test strip in positive assays. For example, home pregnancy dipstick tests detect levels of human chorionic gonadotropin (hCG), a hormone that appears in the blood and urine of pregnant women within days of fertilization. The wick area of the dipstick is coated with mouse monoclonal anti-hCG antibody labeled with colloidal gold, a pink compound (step 1). When the strip is dipped in urine, if hCG is present it will be bound by the pink antibody and the pink hCG-antibody complex will migrate up the strip via capillary action (step 2). When the complex reaches the test zone, a narrow strip containing a fixed unlabeled goat polyclonal anti-hCG, the pink complex will bind and concentrate there, making a pink stripe (step 3). The dipstick tests have a built-in control zone containing polyclonal goat anti-mouse IgG. The unbound pink mouse anti-hCG (present in both positive and negative results) will continue to migrate up the strip, past the test zone, and bind in the control zone, giving a second (or first, if the test is negative for pregnancy) pink stripe (step 4). If no pink stripe appears in the control zone, the test did not function properly.



## Appendix B: Glossary

**3,3',5,5'-tetramethylbenzidine (TMB):** A soluble colorimetric substrate, oxidized to a blue color by horseradish peroxidase and frequently used in ELISA assays.

**Acquired immunity:** A specific response to specific foreign substances that adapts with multiple exposures. Also called adaptive immunity.

**Antibody:** Immunoglobulin protein formed in response to a challenge of the immune system by a foreign agent. Antibodies bind to specific antigens.

**Antigen:** Any agent that provokes an acquired immune response and is bound specifically by either antibodies or T cells.

**Antiserum:** Blood serum containing antibodies raised against a specific antigen.

**Autoimmune disease:** Disease that results from the immune system making a mistake and mounting an immune response against one's own body. Examples are systemic lupus erythematosus (lupus, SLE), rheumatoid arthritis, and multiple sclerosis (MS).

**Bacteriophage:** A virus that infects bacteria; also called a phage. Can be used to introduce foreign DNA into a bacterial genome.

**Chromogenic:** Color-producing. Substrates that produce a colored product when acted upon by an enzyme are termed chromogenic substrates; for example, 3,3',5,5'-tetramethylbenzidine (TMB) produces a blue product when oxidized by horseradish peroxidase.

**Clone:** In the context of molecular biological techniques, "to clone" means to obtain a fragment of DNA from a genome and ligate it into another piece of DNA, such that the ligated DNA now has an identical copy of that gene fragment. In the context of cell biology, "a clone" is a cell or group of cells that are all derived through cell division from the same parent cell and thus have identical genetic data.

**Conjugate:** A substance formed by the covalent bonding of two types of molecules, such as horseradish peroxidase linked to ("conjugated to") an antibody.

**Enzyme:** A protein with catalytic activity. The molecule that an enzyme acts on is called its substrate. Enzymes are classified (and frequently named) on the basis of the reactions that they catalyze. For example, a peroxidase oxidizes its substrate.

**Epitope:** A specific site on an antigen that is recognized by an antibody. Also called antigenic determinant.

**Genetically modified organism (GMO):** An organism whose genetic material (DNA) has been altered in a way that does not occur naturally by mating or natural recombination.

**Horseradish peroxidase (HRP):** An enzyme frequently used to label secondary antibodies. HRP oxidizes substrates (e.g., TMB) for colorimetric detection.

**Immune cell:** Any cell of the immune system, including lymphocytes (B and T cells) and macrophages.

**Immunodeficiency:** Weakening or defects of the immune response such that an individual is unable to mount an effective immune response. May have a genetic basis, result from a disease or other health factor, or be caused by immunosuppressive drugs.

**Immunogen:** Any agent that provokes an immune response. Immunogens that provoke a response from the acquired immune system are called antigens.

**Immunoglobulin (Ig):** General term for all types of antibodies.

**Immunology:** The study of the immune system, the body system that protects the body from foreign substances, cells, and tissues by producing an immune response.

**Innate immunity:** The immunity with which a person is born. Includes cells such as circulating macrophages that respond to foreign invaders. Also called nonadaptive immunity.

**Ligate:** To connect pieces of DNA together, for example, inserting a fragment of an antibody gene into a phage genome.

**Lymphocyte:** Type of white blood cell. Component of the immune system, includes T cells (thymus-derived) and B cells (bone marrow-derived).

**Macrophage:** A type of white blood cell that binds and engulfs foreign materials and antigens in a process called phagocytosis. Macrophages serve two primary functions: 1) removing foreign cells and molecules from the blood; and 2) processing antigens and presenting them on their cell surfaces.

**Microplate:** Molded plastic plate consisting of multiple small wells, usually in a 96-well format.

**Opportunistic infections:** Infections that occur as a result of deficiencies in the immune system, e.g., diseases like oral candidiasis and tuberculosis that occur in immunodeficient AIDS patients.

**Passive immunity:** The acquisition of antibodies from an external source, e.g., antibodies passed from mother to infant, or certain postexposure vaccines such as that for rabies. Passive immunity lasts only a few weeks and does not change with multiple exposures.

**Pathogens:** An organism that can cause disease. Pathogens include bacteria, viruses, fungi, infectious proteins called prions, and parasites.

**Phage display:** A method of producing novel antibodies to specific antigens using recombinant DNA technology and bacterial viruses (bacteriophages).

**Primary antibody:** In an immunoassay, the antibody that binds a specific antigen, conferring specificity to the assay.

**Secondary antibody:** In an immunoassay, the antibody that recognizes the primary antibody, which is from a different species. Secondary antibodies are frequently labeled for easy detection.

**Serum (plural sera):** The clear fluid obtained when the solid components (e.g., red and white blood cells) are removed from whole blood.

**Seroconversion:** The development of detectable antibodies in the blood directed against an infectious agent. For example, after a person is exposed to HIV, no antibodies against HIV can be detected in the blood for approximately 6 weeks. Once seroconversion has occurred, antibodies against HIV can be detected (e.g., by ELISA).

**Subclone:** To transfer an already cloned fragment of DNA into another carrier vector, such as an expression plasmid.

**Substrate:** The target molecule for an enzyme.

**TMB:** see 3,3',5,5'-tetramethylbenzidine.

**Vaccination:** The process of inducing acquired immunity by deliberately stimulating an immune response with a nonpathogenic form of a disease agent. Also called immunization or immunoprophylaxis. The term vaccination originated from the Latin word for cow (*vacca*) because the first vaccination used cowpox to vaccinate against smallpox.

**Vector:** An organism that carries pathogens from one host to another. Vectors are frequently arthropods, e.g., ticks or mosquitoes.

**Zoonosis (plural zoonoses):** An infection transmitted to humans from an animal host, e.g., SARS and rabies.