c0002 CHAPTER 1.2

Immunoassay for Beginners

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p0010 Immunoassays use reagents to generate a signal from minute amounts of target analyte in a sample. Imagine a magnet, securely tied to the end of a fishing line, resting in a stream. Several minute flecks of metal are attracted to the magnet and caught by the person fishing. In an immunoassay, the magnet is replaced by an **antibody**, which is usually immobilized onto a plastic surface instead of a fishing line. Antibodies are very selective and only bind to their specific targets, even in the presence of a huge range of other materials in a sample. As the analytes are present in miniscule concentrations, it is not enough simply to "catch" them to know how much is there. Another reagent has to be used to generate a signal from the captured material. The level of signal indicates the concentration of the specific analyte under test.

p0015 Immunoassays derive their unique specificity, sensitivity, and flexibility from three important properties of antibodies:

u0010 • Their ability to bind to an extremely wide range of natural and man-made chemicals, biomolecules, cells, and viruses.

u0015 • Exceptional specificity for the substance to which each antibody binds.

u0020 • The strength of the binding between an antibody and its target.

p0035 Antibodies can be generated by vaccinating animals with the analyte of interest. This process is described as **immunization**.

Immunometric Immunoassays

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p0040 The simplest type of immunoassay to understand is the **immunometric** design (Fig. 1). An antibody immobilized onto a plastic surface (such as a well in a microtiter® plate) **captures** the test analyte from the sample, and a different antibody, specific for another part of the analyte molecule, is used as the basis of the **signal generation system**. This antibody is "labeled," e.g., with a radioactive isotope. After an incubation to allow the antibodies to bind with the analyte, unbound labeled antibody is washed away. The final stage of the assay involves measurement of the level of signal, which is radioactivity in this example. The signal level in this type of assay is proportional to the analyte concentration in the sample.

p0045 The labeled component of an immunoassay is sometimes called the **tracer**. The efficient removal of unbound tracer by washing is a critical part of the assay, known as the **separation**. The material (normally plastic) that the capture antibody is irreversibly bound to is known as the **solid phase**. Because the antibodies form a sandwich around the analyte, immunometric assays are also known as **sandwich** assays.

p0050 In the next example the format is similar, but the immunoassay has been designed to detect *antibodies* in a blood sample using the appropriate antigen as the "bait." This application is useful for detecting previous exposure to a specific infectious disease. Proteins that occur on the surface of a virus can be immobilized onto plastic. They

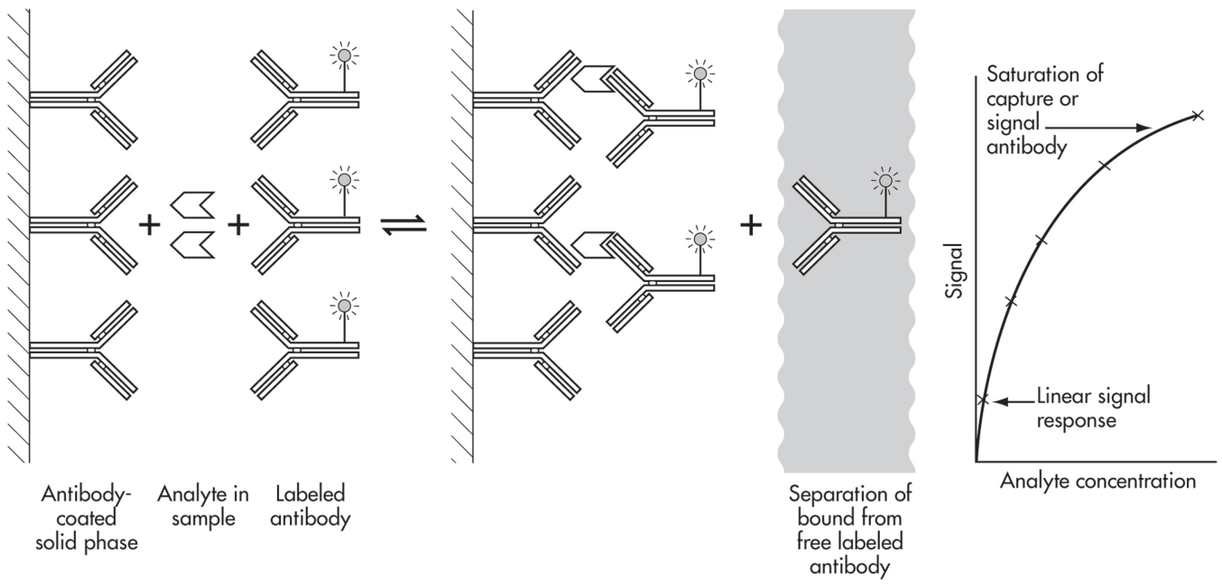

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FIGURE 1 Immunometric immunoassay.

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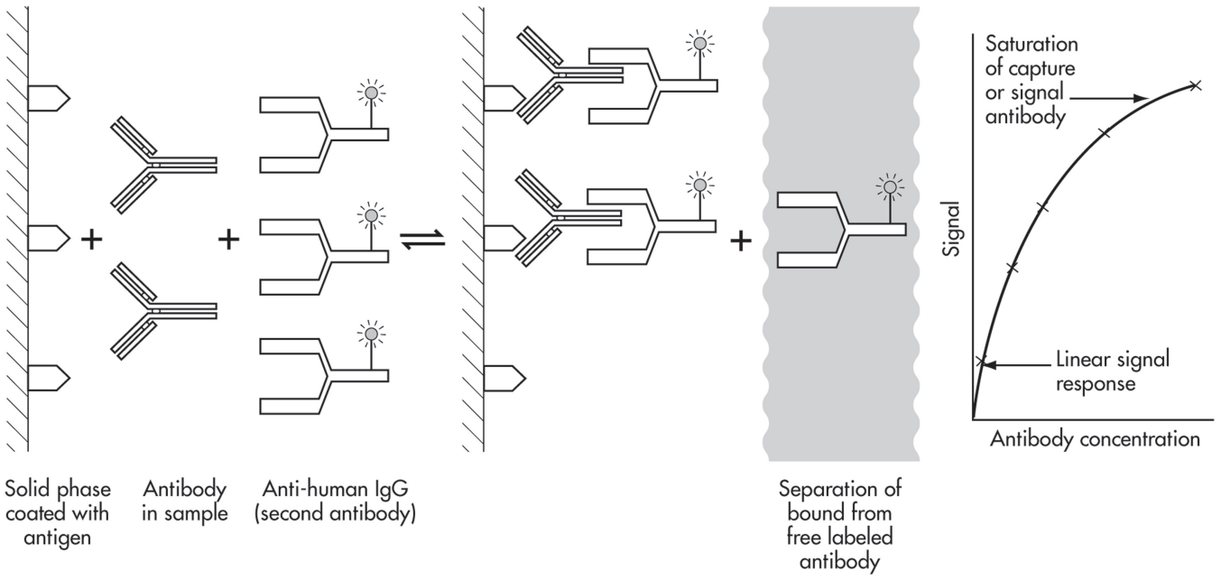

FIGURE 2 Immunometric assay for antibody testing.

capture specific antibodies for that virus from the sample. As a tracer, a labeled antibody raised in animals against the constant region of human antibody can be used. This is sometimes referred to as the **second antibody** (Fig. 2).

p0055 The next figure shows a similar assay with a different type of label used for the tracer (Fig. 3). Instead of a radioactive isotope, an **enzyme** is chemically attached (**conjugated**) to the labeled antibody. Like antibodies, enzymes are proteins that bind to specific targets, but enzymes also catalyze specific reactions. The starting material for an enzyme-catalyzed reaction is called a **substrate**. Enzyme labels, with the appropriate substrate, can be used to generate color or create fluorescent or luminescent end products, which can be readily measured by optical and electronic equipment. Each molecule of enzyme can convert many molecules of substrate, providing a sensitive signal generation system. This format of assay is often known as an **enzyme-linked immunosorbent assay (ELISA)**.

s0015 Competitive Immunoassays

p0060 Immunometric assays work well when the target analyte is a large molecule with sufficient surface area to accommodate two molecules of antibody. However, many immunoassays are for small molecules such as drugs, and a different design is needed. This is illustrated in the next figure (Fig. 4). Only one antibody is used, and it is present in a limited quantity. The other key reagent, the tracer, is made from the target analyte, labeled with a suitable signal generation material, such as a radioisotope or enzyme. The proportion of tracer that binds to the limited antibody sites is *indirectly* proportional to the concentration of analyte in the sample. This is known as a **competitive** immunoassay. In this type of assay, the exact amounts of immobilized antibody and labeled analyte are critical and these assays are sometimes referred to as **reagent limited**. (In the same context, immunometric (sandwich) assays are described as **reagent excess**.)

p0065 In immunoassays, the analyte that the antibodies bind to is often referred to as the **antigen**, although the word "antigen" refers to a substance capable of *provoking* an antibody response. In many competitive immunoassays, the analyte molecules are too small to elicit an antibody response in animals and need to be chemically linked (conjugated) to a larger molecule, usually a protein, to generate antibodies. Once the antibodies have been generated, it is usually possible to find antibodies from some or all of the animals vaccinated that bind to the analyte alone. In this situation, the analyte is referred to as a **hapten**. The molecule used to immunize the animals, whether it is the pure analyte or a conjugated version, is called the **immunogen**.

s0020 Homogeneous Immunoassays

p0070 So far, each type of immunoassay described has depended on a separation of unbound tracer before the bound signal is measured. Without a separation (such as a thorough aspiration and wash of the solid phase with buffer prior to signal generation) the level of signal would always be the same, regardless of the concentration of analyte. These assay formats are all examples of **heterogeneous** immunoassay. Some assays have been developed that do not require a separation, in which the tracer only generates the signal when it binds to the analyte in an immunometric assay or to the antibody in a competitive assay. They are known as **homogeneous** immunoassays (Fig. 5).

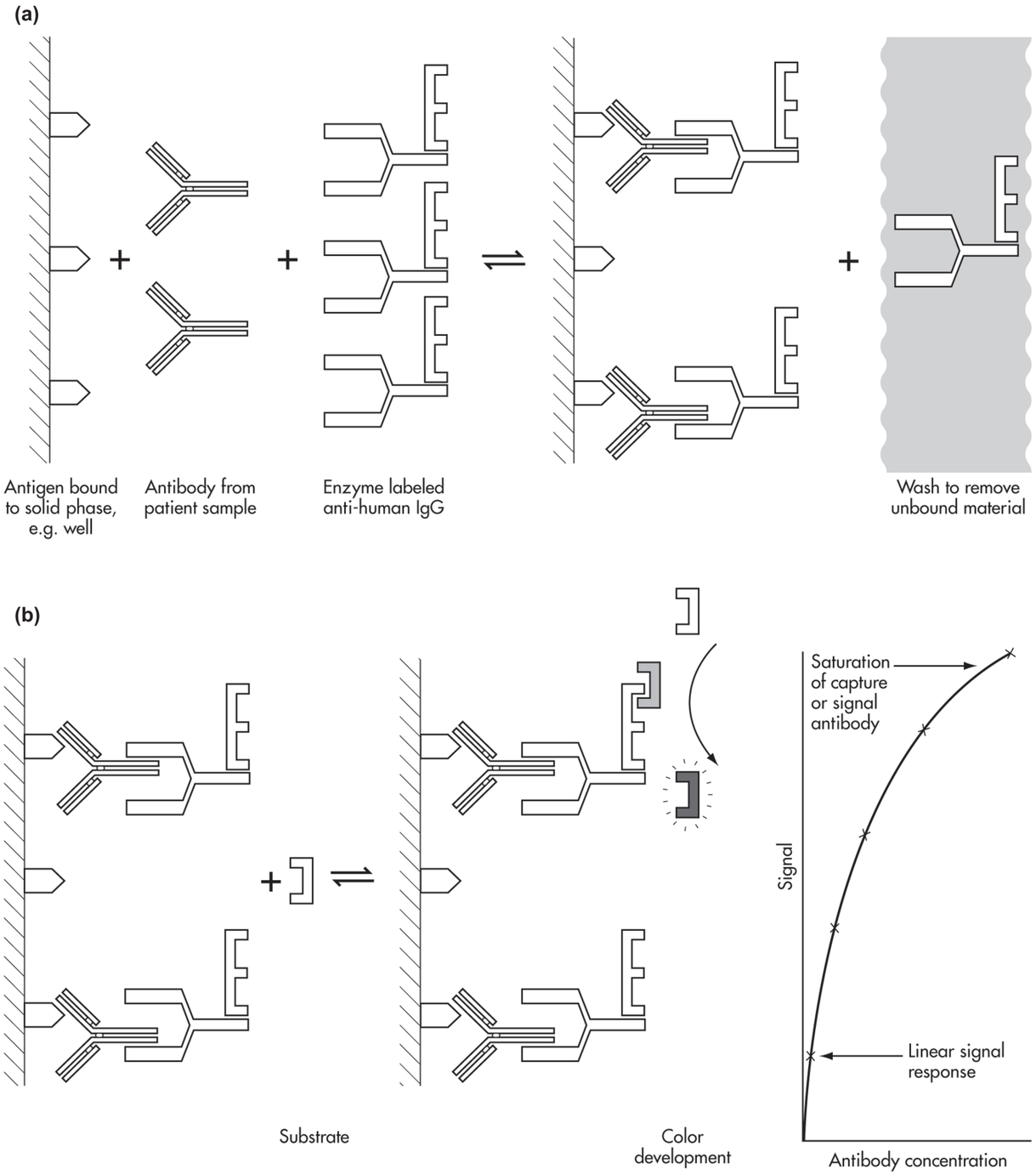

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FIGURE 3 Enzyme immmunoassay for detection of antibodies (ELISA).

s0025 CALIBRATION

p0075 In order to estimate the concentration of an analyte from the signal generated, a **standard curve** is required, which is created by including dilutions of a solution with a known concentration of analyte in the same assay as the unknown samples. In commercial assay kits, the curve is usually generated by the user from a pre-calibrated set of solutions, known as **calibrators**, and the curve generated using them is called the **calibration curve**.

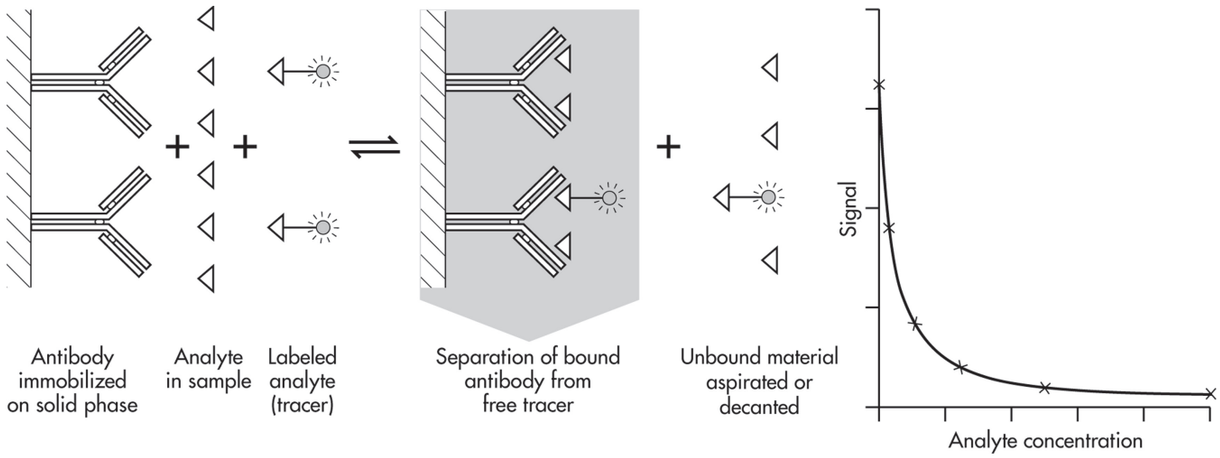

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FIGURE 4 Competitive immunoassay (solid phase separation).

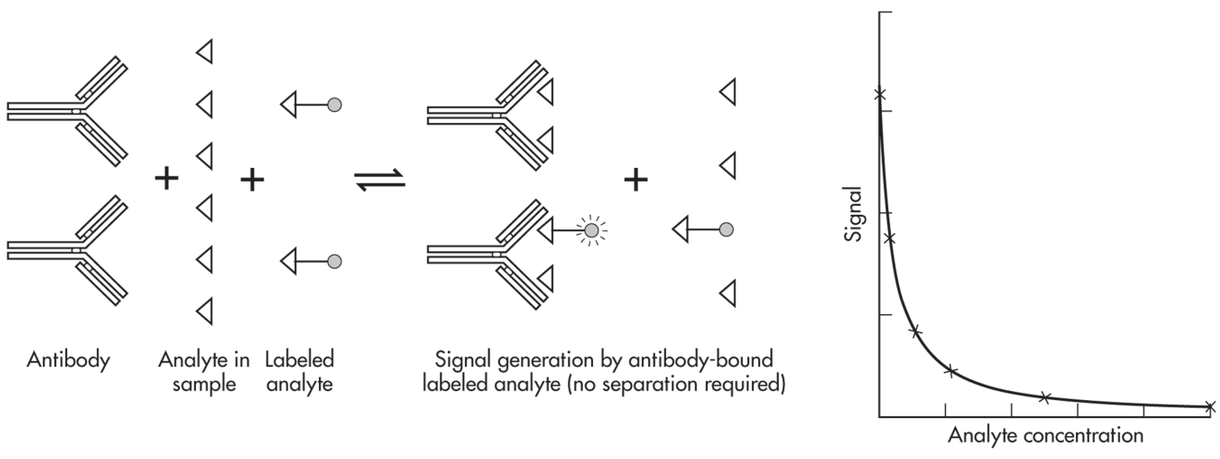

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FIGURE 5 Example of homogeneous immunoassay.

p0080 Immunoassays that measure analyte concentrations against a standard or calibration curve are **quantitative**. But some immunoassays have been simplified to perform a single task, such as indicating if a urine sample is from a pregnant or non-pregnant woman. Such tests are known as **qualitative**, and pregnancy tests available to the general public are a well-known example.

CONCLUSION

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p0085 For a deeper understanding of immunoassays, the reader is referred to the first chapter, HOW TO USE THIS BOOK, to find out where information on immunoassay formats, components, and applications is located.