INTRODUCTION

Immunoassays encompass techniques for the detection and quantification of antigens or antibodies, and are one of the most powerful of all immunochemical techniques. Although the term “immunoassay” generally refers to a quantitative method, in a much broader sense it also includes characterizing methods for analyzing the immunological properties of analytes.

The nomenclature of immunoassay systems is at times quite confusing. This is at least in part due to several variations that exist in the design of particular immunoassays. Generally, all assay names contain the word “immuno” combined with another word indicating the type of label used, along with the word “assay.” For example, radioimmunoassays (RIAs) describe systems in which the detection label is a radioisotope. Radioreceptor assays are analogous systems except that the antibodies are replaced with specific receptors.

In contrast, in nonisotopic immunoassays, none of the reactants is labeled with a radioisotope. In all such systems, a variety of markers or labels, individually or in combination, are used to follow and measure the assay reactions. Examples of this type include enzyme immunoassays (or enzymoimmunoassay, EIA), fluorescent immunoassay (or fluoroimmunoassay), and chemiluminescent immunoassay (or chemiluminoimmunoassay).

When the immunoassay involves the use of reagents in stoichiometric excess, the nomenclature involves the words “immuno” and “metric.” In general, the term “immunometric assay” is used to describe reagent excess assays. Thus, according to the label used, such an assay may be designated as an immunoradiometric assay (IRMA), immunoenzymometric assay, or immunofluorometric assay.
The term “enzyme-linked immunosorbent assay” (ELISA), first coined by Engvall and Perlmann (1971), is generally used for reagent-excess enzyme immunoassays of specific antibodies or antigens. When the ELISA is applied to antigens, it is the equivalent of the IRMA. However, in the diagnostics industry, the term ELISA is often used interchangeably with enzyme immunoassay (or enzymo-immunoassay) and immunoenzymometric assay.

In this chapter, the more commonly used classification systems for describing immunoassays, the various assay designs and formats, and important commercial technologies built around enzyme immunoassays are described.

### CLASSIFICATION

Immunoassays are performed in several different ways, and, therefore, can be classified on the basis of many different criteria. Some of these include the type of analysis (quantitative or qualitative), test sample (antigen or antibody), assay system (labeled versus nonlabeled, competitive versus noncompetitive, separation free versus separation required, and end-point detection either visibly or by instrumentation), and assay conditions (liquid versus solid phase, equilibrium versus nonequilibrium, manual or automated).

One of the earliest classification systems divided immunoassays as nonlabeled or labeled reagent assays. The nonlabeled immunoassays, because of a lack of signal amplification system, have limited sensitivity, because large antigen:antibody complexes must be formed for their detection. These include immunoprecipitin and agglutination methods and the corresponding light-scattering techniques (e.g., nephelometry and turbidimetry) for the detection of antigen:antibody complexes either by equilibrium or by kinetic analyses. These methods with sensitivity in the range of micromoles per liter are generally used for analyzing proteins.

### Fundamental Assay Designs

As described earlier in Chapter 3, the basic principle of an immunoassay is a reversible reaction between an antigen (Ag) and its antibody (Ab) as follows:

$$\text{Ag} + \text{Ab} \xrightarrow{k_1} \text{Ag : Ab} \xleftarrow{k_2} \text{Ag} + \text{Ab}$$

The antigen combines with the antibody to form the Ag:Ab complex at rate constant $k_1$. At equilibrium, the complex dissociates with a rate constant $k_2$ to form free Ag and Ab. The term antigen in the following discussion is used interchangeably with analyte or ligand.

Based on this principle, immunoassays may be broadly divided into two classes (Ekins 1981, 1985; Nakamura et al. 1986) as follows: