
Preface

The aim of *The ELISA Guidebook* is to expand the information concerning enzyme-linked immunosorbent assay (ELISA) published in *ELISA: Theory and Practice* by J. R. Crowther (1995), in the *Methods in Molecular Biology* series by Humana Press (vol. 42). The earlier book concentrated on the immunological background of the reagents exploited in such assays, and dealt practically with the various assays, through examples using noninfectious systems. This new volume is a major extension and updating of that book, with a reorganization of the chapters, and extra information dealing, in particular, with chess-board titration of reagents, quality control, monoclonal antibodies, validation of assays, statistics, and epidemiological considerations. Suitable for scientists with previous experience of the technique, it can, however, be used successfully by those with little experience, and as a teaching aid.

The ELISA Guidebook deals with heterogeneous enzyme-linked immunosorbent assays. The abbreviation ELISA, or in the plural ELISAs, will be used from now on to denote this kind of assay. Besides the inherent feature of all ELISAs—that there is an enzyme linked to one of the reagents—heterogeneous assays involve the attachment of one reagent to a solid phase and subsequent addition of reagents that bind. The separation of bound and free components is necessary through washing steps. Such assays must be distinguished from homogeneous ELISAs, in which reagents are added simultaneously.

ELISAs remain the mainstay of testing in which the specificity inherent in antibodies is exploited. The technique is still expanding in all fields of pure and applied biology, and in particular, now constitutes a backbone diagnostic technique. Recent applications into quality assessment of foods for contaminants is testimony to the flexibility for these possible systems. There is an increasing use of automated systems in commercial applications of ELISA; however, there is still a major use for more manual techniques in the development of assays, and for routine use in laboratories with lesser facilities. A thorough understand-

ing of the principles is vital to the proper use of ELISA, even where established kits are provided.

The key to all ELISA systems is the use of antibodies. These are proteins produced in animals in response to antigenic stimuli. Antibodies are specific chemicals that bind to the antigens used for their production; thus, they can be used to detect particular antigens if binding can be demonstrated. Conversely, specific antibodies can be measured by the use of defined antigens, and this forms the basis of many assays in diagnostic biology.

Besides covering the various assay parameters, the basic reagents, and the skills needed to perform ELISA, *The ELISA Guidebook* introduces these increasingly important topics: quality control of testing; kit production; validation; statistical requirements for examination of data and for epidemiological studies; equipment choice, care, and calibration; technology transfer; and monoclonal antibodies. Wherever possible, explanations are provided in diagrammatic, as well as written, form. The text may, in places, seem repetitious. However, in the experience of the author, and through feedback from the previous publication, readers respond very differently to various approaches, so that conveying information by multiple exposures is considered pedagogically useful.

Although often reviewed, it is worth considering the beginnings of ELISA, which stemmed from investigations of the ability of enzyme-labeled antibodies (1–3) to identify antigens in tissue. The methods of conjugation were exploited to measure serum components in the first “true” ELISAs (4–6).

By far the most exploited ELISAs use plastic microtiter plates in an 8 X 12 well format as the solid phase (7). Such systems benefit from a large selection of specialized commercially available equipment including multichannel pipets for the easy simultaneous dispensing of reagents and multichannel spectrophotometers for rapid data capture. There are many books, manuals, and reviews of ELISA and associated subjects that may be examined for more practical details (8–21). The following table summarizes some of the features that make ELISA so sustainable a technique.

Advantages of ELISA

1. Simplicity	(a) Reagents added in small volumes (b) Separation of bound and free reactants is made by simple washing procedures (c) Passive adsorption of proteins to plastic is easy (d) Specialized equipment readily available
2. Reading	(a) Colored end-product can be read by eye to assess whether tests have worked (avoiding waiting for results where machine reading essential as in RIA) (b) Multichannel spectrophotometers quantify results that can be examined statistically
3. Rapidity	(a) Tests can be performed in a few hours (b) Spectrophotometric reading of results is rapid (96 wells read in 5 s)
4. Sensitivity	Detection levels of 0.01 to 1 $\mu\text{g/mL}$ are easily and consistently achievable. These levels are ideal for most diagnostic purposes
5. Reagents	Commercially available reagents offer great flexibility in ELISA design and achievement of specific assays
6. Adaptability	Different configurations allow different methods to be examined to solve problems. This is useful in developing tests and research science
7. Cost	(a) Startup costs are low (b) Reagent costs are low
8. Acceptability	Fully standardized ELISAs in many fields are now accepted as "gold-standard" assays
9. Safety	Safe nonmutagenic reagents are available. Disposal of waste poses no problem (unlike radioactivity)
10. Availability	ELISAs can be performed anywhere, even in laboratories where facilities are less than state of the art
11. Kits	ELISA kits are widespread and successful
12. Standardization	Quantification of data allows easier standardization

All the key elements listed will be examined in detail in this book. The background needed in immunologic/serologic aspects is not dealt with extensively as a discrete chapter, rather points are included at appropriate times. Scientists involved in developing and using ELISA should be familiar with the concepts inherent in immunology. There are several excellent textbooks, including Roitt and colleagues (22), that should be read. Immunochemical methods are also important, e.g., in purifying and exploiting antigens and antibodies, and for conjugat-

ing proteins. An excellent manual covering all aspects of immunochemistry is available [Harlow and Lane (23)], which also outlines many relevant laboratory practices.

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