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## Preface

There have been very few developments that markedly affect the need to greatly revise the text from the last version of this book. This is testament to the fact that heterogeneous enzyme-linked immunosorbent assays (ELISA) provide ideal systems for dealing with a wide range of studies in many biological areas. The main reason for this success is test flexibility, whereby reactants can be used in different combinations, either attached passively to a solid phase support or in the liquid phase. The exploitation of the ELISA has been increased through continued development of specifically produced reagents, for example, monoclonal and polyclonal antibodies and peptide antigens coupled with the improvement and expansion of commercial products such as enzyme-linked conjugates, substrates and chromogens, plastics technology and design of microwell plates, instrumentation advances and robotics. However, the principles of the ELISA remain the same. There has been some rearrangement of chapters plus addition of three new ones dealing with charting methods for assessing the indirect ELISA, ruggedness and robustness of tests-aspects of kit use and validation, and internal quality control and external quality management of data, respectively. These reflect the need to control what you are doing with ELISA and to exploit the method to its full extent. I do not apologize for dealing with the same areas in different ways a number of times, as it is imperative that principles are understood to allow planning, operation, and control of ELISA.

A brief scan of the literature involving ELISA can be used to illustrate the continued success of ELISA. The number of publications with ELISA mentioned in all science areas from 1976 to 2004 is shown in Table 1. A fairly constant increase in the number of research works using ELISA methods is indicated. A breakdown of publications according to the areas of science in 5 yearly periods from 1980 given in Table 2 illustrates the versatility in the use of ELISA, as well as highlights the major areas of use in medicine and dentistry; immunology and microbiology, molecular biology, and genetics and biotechnology. It is interesting to note that the earliest exploitation of ELISA was in immunology and microbiology and molecular Biology and biotechnology, probably reflecting the greatest research areas. Medicine and dentistry (associated by the search engine) shows the greatest rate of increase in use (probably in the medical sphere only) from the 1990s.

The search results indicate the continued expansion of ELISA in science, and there is no reason to believe that this will change even in the face of modern technologies exploiting molecular methods. The analytical and systematic characteristics of ELISA are ideally suited to diagnosis at the screening level, for surveillance where larger scale sample handling is required, and for research. Many of the accepted standard assays in many scientific fields are ELISA-based and have replaced other “gold standard” assays. In conjunction with the rapidly evolving use of molecular methods centering on the polymerase chain reaction (PCR) technologies, there is a need to use serological confirmatory methods in a dual approach to directly identify and characterize disease agents and to assess disease prevalence through the measurement of specific antibodies or other chemical factors as a result of infection. The use of ELISA methods in testing the environment and animal or plant products as safe for human and animal consumption is also a rapidly evolving area for ELISA.

**Table 1**  
**Literature search in ScienceDirect database for ELISA**

Year	Number
1976	6
1977	13
1978	14
1979	31
1980	45
1981	95
1982	125
1983	216
1984	257
1985	367
1986	420
1987	547
1988	565
1989	640
1990	682
1991	743
1992	774
1993	820
1994	870
1995	1,016
1996	1,093
1997	1,119
1998	1,099
1999	1,144
2000	1,118
2001	1,120
2002	1,198
2003	1,253
2004	1,591

ELISA, therefore, has been used in all fields of pure and applied aspects of biology. In particular, it forms the backbone of diagnostic techniques. The systems used to perform ELISAs make 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 the 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.

**Table 2**  
**Breakdown of literature search in science groups**

Subject	1980–1984	1985–1989	1990–1994	1995–1999	2000–2004
Agriculture and biological sciences	87	274	615	804	827
Molecular biology, genetics, and biotechnology	374	1,329	1,762	1,845	2,096
Chemistry	8	29	77	208	279
Environmental science	4	13	52	125	162
Immunology and microbiology	514	1,584	2,128	2,450	2,772
Medicine and dentistry	280	971	1,639	2,875	3,372
Neurosciences	21	124	198	380	484
Pharmacology and toxicology	24	108	247	397	497
Veterinary sciences	71	219	522	769	853

The book describes the methods involved in ELISAs, where one of the reagents, usually an antibody, is linked to an enzyme and where one reagent is attached to a solid phase. The systems allow the examination of reactions through the simple addition and incubation of reagents. Bound and free reactants are separated by a simple washing procedure. The end product in an ELISA is the development of color, which can be quantified using a spectrophotometer. These kinds of ELISA are called heterogeneous assays and should be distinguished from homogeneous assays where all reagents are added simultaneously. The latter assays are most suitable for detecting small molecules such as digoxin or gentamicin.

The development of ELISA stemmed from investigations of enzyme-labeled antibodies (1–3), for use in identifying 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 microtitre plates in an 8 × 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, which should be examined for more detailed practical details (8–21).

The purpose of developing ELISAs is to solve problems. These can be divided into pure and applied applications, although the two are interdependent. Thus, a laboratory with a strong research base is essential in providing scientific insight and valuable reagents to allow more routine applications. The methods outlined show the flexibility of the systems. Their effective use is up to the ingenuity of scientists. Recent advances in science have given the immunoassayist greater potential for improving the sensitivity and specificity of assays, including ELISA. In particular the development of MAb technology has given us single chemical reagents (antibodies) of defined specificity, which can be

standardized in terms of activity as a function of their weight. The development of gene expression systems has also given the possibility of expressing single genes as proteins for use in raising antibodies or acting as pure antigens. This technology goes hand-in-hand with developments in the polymerase chain reaction (PCR) technologies, which enables the very rapid identification of genes and their manipulation. In turn, improvements in the fields of rapid sequencing and X-ray crystallographic methods has led to a far more intimate understanding of the structure–function relationship of organisms in relation to the immunology of disease. The ELISA fits in rather well in these developments, since it is a binding assay requiring defined antibodies and antigens, all of which can be provided. Table 3 illustrates some applications of ELISA with relevant references.

**Table 3**  
**Applications of ELISA**

General	Specific	References
Confirmation of clinical disease	Titration of specific antibodies	(21–35)
	Single dilution assays	(27, 30–34, 36)
	Relationship of titer to protection against disease	(29, 37)
	Kits	(28, 32, 33)
Analysis of immune response to whole organisms, purified antigens extracted from whole organisms, expressed proteins (e.g., vaccinia, baculo, yeast, bacteria), measurement, polypeptides, peptides	Antibody quantification	(25, 26, 32, 34, 36, 38–40)
	Antibody class measurement (IgM, IgG, IgA, IgD, IgE)	(41–44)
	Antibody subclass measurement (IgG1, IgG2b, IgG3)	(42)
	Antibody IG2a, affinity	(28, 45, 46)
Antigenic comparison	Relative binding antibodies	(25, 26, 34, 40, 47)
	Affinity differences in binding of antibodies	(40, 45, 48–50)
	Measurement of weight of antigens	(28, 34, 46, 48, 51–56)
	Examination of treatments to antigen (inactivation for vaccine manufacture, heating, enzyme treatments)	(46)

(continued)

**Table 3**  
**(continued)**

General	Specific	References
	Identification of continuous and discontinuous epitopes by examination of binding of polyclonal and MAbs to denatured and nondenatured proteins	(28, 55, 57, 58)
	Antigenic profiling by MAbs	(28, 57, 59–61)
	Comparison of expressed and native problems	(5, 55, 62, 63)
	Use of MAbs to identify paratopes in polyclonal sera	(58, 62, 64)
Monoclonal antibodies	Screening during production	(57, 59)
	Competitive assay-antibody assessment	(62)
	Comparison of antigens	(28, 32, 57, 58, 60, 62)
	Use of MAbs to orientate antigens	(55)
Novel systems	High-sensitivity assays (Amplified-ELISA)	(65)
	Fluorogenic substrates	(66)
	Biotin–avidin systems	(67)
More recent references	Food analysis	(68–70)
	Fish	(71–75)
	AIDS	(75–77)
	SARS	(78)
	Bird flu	(79, 80)
	Allergens	(81, 82)
	Emerging diseases	(83)
	Psychiatry	(84)
	Review	(85, 86)
	Snakes	(87)
	Environment	(88)
	Chemoluminescence	(89)

The ability to develop ELISAs depends on as closer understanding of the immunological/serological/biochemical knowledge of specific biological systems as possible. Such information is already available with reference to literature surveys. Basic skills in immunochemical methods are also a requirement and an excellent manual for this is

available (90). References (91, 92) are excellent text books on immunology. An invaluable source of commercial immunological reagents is available in (69). The references from 70 onwards are more recent and reflect newer fields into which ELISA has expanded and also the new problems arising as, for example, Avian influenza and SARS. It is difficult to see that there will be a significant reduction in the rate of use of ELISA directly or as part of other molecular systems, but this can only be assessed when the next edition of this book is written. The main danger is methods involving ELISA are now regarded easy to develop. This, as for all tests, is not true and good training in ELISA is even more important nowadays, since there is an incredible spectrum of reagents available for the development of tests. The linking of molecular methods to ELISA and other detection systems based on solid phase assays is exciting and full of potential, but there is a great need to attend to the basic understanding and principles of ELISA.

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