Contents

Preface — v Acknowledgments — vii			
1	Introduction —— 1		
1.1	The History, contents and theories in histochemistry —— 1		
1.1.1	Origin and development of histochemistry —— 1		
1.1.2	Contents of histochemistry studies —— 2		
1.1.3	Theories of histochemistry —— 2		
1.2	Basic requirements of histochemistry methods — 3		
1.2.1	Basic requirements —— 3		
1.2.2	Things you need to know —— 4		
2	Tissue preparation —— 5		
2.1	Tissue collection — 5		
2.1.1	Attention — 5		
2.1.2	Size of specimen — 5		
2.2	Fixation —— 5		
2.2.1	Purpose of fixation —— 5		
2.2.2	Object for fixation —— 6		
2.2.3	Quality of the fixative —— 6		
2.2.4	Methods for tissue fixation —— 6		
2.2.5	Notes for fixation —— 7		
2.2.6	Frequently used fixative — 7		
2.3	Tissue rinse, dehydration and clearance —— 14		
2.3.1	Rinse 14		
2.3.2	Dehydration —— 14		
2.3.3	Clearing —— 14		
2.4	Embedding —— 15		
2.4.1	Embedding medium for light microscopy technology —— 15		
2.4.2	Embedding medium for electron microscopy technology —— 15		
2.5	Sectioning —— 16		
2.5.1	Paraffin section method —— 16		
2.5.2	Frozen section method —— 16		
2.6	Adherence and mounting —— 17		
2.6.1	Adherence and adhesive medium —— 17		
2.6.2	Mounting and mounting medium —— 18		
2.7	Buffer —— 19		



2.7.1	Composition and application of buffer —— 19
2.7.2	Commonly used buffer —— 20
3	Carbohydrate and its derivatives in histochemistry —— 21
3.1	Classification —— 21
3.1.1	Chemistry of carbohydrates —— 21
3.1.2	Histochemical classification of carbohydrates —— 22
3.2	Histochemistry methods —— 23
3.2.1	Technology for the demonstration of glycogen —— 23
3.3	Display method of glycoconjugates (mucins) —— 27
3.3.1	Alcian blue staining acidic glycoconjugates —— 27
3.3.2	Mast cells with toluidine blue stain —— 29
4	Nucleic acid histochemistry —— 31
4.1	DNA histochemical demonstration —— 31
4.1.1	Feulgen reaction —— 31
4.1.2	The improved Feulgen reaction —— 32
4.2	Comparison demonstration of DNA and RNA —— 33
4.2.1	Methyl green-pyronin stain —— 33
4.2.2	Acridine orange stain —— 34
5	Lipid histochemistry —— 37
5.1	Overview of the lipid histochemistry —— 37
5.1.1	Categories of lipids —— 37
5.1.2	Fixation —— 37
5.2	Demonstration of lipids with physical methods —— 38
5.2.1	Neutral lipids demonstration by Sudan black stain —— 38
5.2.2	Neutral lipids demonstration by Oil Red O stain —— 39
5.3	Demonstration of lipids with chemical methods —— 39
5.3.1	Theory —— 40
5.3.2	Materials and reagents —— 40
5.3.3	Procedure —— 40
5.3.4	Results —— 40
5.3.5	Notes —— 40
6	Enzyme histochemistry —— 43
6.1	Enzyme and its basic histochemical theory —— 43
6.1.1	Classification of biological enzymes in body —— 43
6.1.2	Significance of enzyme histochemistry —— 43
6.1.3	Histochemical reaction of enzyme —— 43
6.1.4	Influencing factors of displaying effects in enzyme histochemistry method —— 46

6.2	Histochemistry for common enzymes —— 47
6.2.1	Alkaline phosphatase (AKP) —— 47
6.2.2	Acid phosphatase (ACP) —— 50
6.2.3	Succinate dehydrogenase (SDH) —— 52
6.2.4	Lactate dehydrogenase (LDH) —— 54
7	Basic theory of immunohistochemistry —— 57
, 7.1	Basic immunology —— 57
7.1.1	Antigen —— 57
7.1.2	Antibody —— 58
7.2	Common markers and their detection —— 61
7.2.1	Fluorescent dyes —— 61
7.2.2	Enzyme —— 62
7.2.3	Biotin —— 65
7.2.4	Colloidal gold —— 66
7.3	Basic conditions —— 67
7.3.1	Storage of antigens and unmasking of antigens —— 67
7.3.2	Specific antibody — 70
7.3.3	Reagents —— 71
,	10030110 /1
8	Commonly used methods in immunohistochemistry —— 77
8.1	Theories of different methods —— 78
8.1.1	Direct method —— 78
8.1.2	Indirect method —— 78
8.1.3	Unlabeled antibody enzymatic method —— 78
8.2	Immunofluorescence method —— 80
8.2.1	Theories —— 80
8.2.2	Staining methods —— 81
8.2.3	Control experiments —— 82
8.2.4	Observing and recording of the results —— 82
8.2.5	The elimination of nonspecific fluorescence —— 83
8.3	Immunoenzyme method —— 84
8.3.1	Theory —— 84
8.3.2	Common methods —— 84
8.3.3	PAP method —— 85
8.4	Avidin-biotin method —— 87
8.4.1	Theory —— 87
8.4.2	ABC method —— 88
8.4.3	SP method —— 91
8.5	Protein A method —— 92
8.5.1	Nature of the protein A and its applications —— 92
8.5.2	Staining method —— 92

8.6	Immunogold and immunogold-silver method —— 93
8.6.1	Immune colloidal gold method —— 93
8.6.2	Immunogold-silver method —— 95
9	Specificity and sensitivity of immunohistochemistry —— 97
9.1	Specificity and immunohistochemistry staining — 97
9.1.1	Specificity —— 97
9.1.2	Specific and nonspecific staining —— 97
9.2	Control experiment —— 98
9.2.1	Positive controls — 98
9.2.2	Negative controls —— 99
9.3	Methods to enhance the immunohistochemical sensitivity — 100
9.3.1	Enhanced specific staining —— 100
9.3.2	Causes of background staining and the methods of
	elimination —— 102
10	Double-staining immunohistochemistry technology —— 105
10.1	Double-staining immunohistochemistry on serial sections —— 105
10.2	Immunofluorescence double-staining technology —— 105
10.2.1	Direct immunofluorescence double-staining technology —— 106
10.2.2	Indirect immunofluorescence double-staining technology —— 106
10.3	Immunoenzyme double-staining technology —— 107
10.3.1	Basic concept —— 107
10.3.2	Antibody elution —— 108
10.3.3	Immunohistochemistry double staining with primary antibodies
	from different species —— 111
10.3.4	Immunohistochemistry double staining of primary antibodies
	from the same species —— 112
10.4	Immunoenzyme-immunofluorescence double staining —— 114
10.5	Immunoenzyme-immunogold double staining —— 114
11	Lectin histochemistry —— 115
11.1	Characteristics and application of lectin —— 115
11.1.1	Structure of carbohydrate in the tissue —— 115
11.1.2	Characteristics of lectins —— 115
11.1.3	Application of lectins —— 116
11.2	Application of lectin histochemistry —— 117
11.2.1	Principle —— 117
11.2.2	Direct method —— 118
11.2.3	Antibody method —— 119
11.2.4	Biotin-labeled method —— 121
11.2.5	Carbohydrate-lectin-carbohydrate sandwich method —— 122
11.2.6	Control test —— 123

12	Progresses of <i>in situ</i> display —— 125
12.1	Envision method —— 125
12.2	Catalyzed signal amplification method —— 125
12.3	Ferric oxide alternative method for HRP —— 126
13	<i>In situ</i> hybridization histochemistry technology —— 127
13.1	Basic theory —— 127
13.2	Probe preparation —— 127
13.2.1	Category of probes —— 128
13.2.2	Principal and method of probe labeling —— 128
13.3	Procedure of ISHH —— 129
13.3.1	Basic procedure —— 129
13.3.2	Detection of mRNA on paraffin-embedded sections with
	DNA probes —— 129
13.3.3	Detection of mRNA on paraffin-embedded sections with DIG-labeled
	RNA probes —— 139
13.4	Factors affecting <i>in situ</i> hybridization —— 142
13.4.1	Concentration of probe —— 142
13.4.2	Hybridization temperature —— 143
13.4.3	Hybridization time —— 143
13.4.4	Hybridization buffer —— 143
13.5	Control test —— 144
13.5.1	Tissue control —— 144
13.5.2	Probe control —— 145
13.5.3	Hybridization reaction control —— 145
13.5.4	Detection system control —— 146
14	<i>In situ</i> polymerase chain reaction histochemical technology —— 147
14.1	Basic theory —— 147
14.2	Basic types —— 147
14.2.1	Direct ISPCR —— 147
14.2.2	Indirect ISPCR —— 147
14.2.3	In situ reverse transcription PCR (RT-PCR) —— 147
14.3	Procedures —— 148
14.3.1	Tissue preparation —— 148
14.3.2	ISPCR —— 148
14.3.3	<i>In situ</i> detection —— 149
14.3.4	Procedure of direct ISPCR —— 149
14.3.5	Procedure of indirect ISPCR —— 150
14.3.6	Control test —— 150
14.4	Application of ISPCR technology —— 150
14.4.1	Detection of exogenous gene —— 151
14.4.2	Detection of endogenous gene —— 151

15	Electron microscopic histochemistry technology —— 153	
15.1	Electron microscopic enzyme histochemistry technology —— 153	
15.1.1	Procedures of electron microscopic enzyme histochemistry — 153	
15.1.2	Examples of electron microscopic enzyme histochemistry —— 157	
15.1.3	Electron microscopic enzyme histochemistry of organelles —— 160	
15.2	Electron microscopic immunohistochemistry technology —— 163	
15.2.1	Technical requirements of immunoelectron microscopy —— 164	
15.2.2	Immunoenzyme electron microscopic technology — 166	
15.2.3	Immunogold electron microscopic technology —— 170	
15.2.4	The double immunostaining transmission electron microscope	
	technology —— 172	
15.2.5	Immunostaining scanning electron microscope technology —— 175	
	Quantitative assay of histochemistry experiment results —— 179	
16.1	Photomicrography —— 179	
16.1.1	Basic theory —— 179	
16.1.2	Application — 180	
16.2	Image analysis —— 180	
16.2.1	Theories of image analyzer —— 181	
16.2.2	Working procedures of image analyzer —— 181	
16.2.3	Application of image analyzer in biomedical research —— 182	
16.3	Flow cytometry —— 182	
16.3.1	Main structure and basic theory of FCM —— 183	
16.3.2	Sample preparation for FCM —— 184	
16.3.3	Application of FCM —— 186	
16.4	Laser scanning confocal microscopy —— 187	
16.4.1	Theory and characteristic of LSCM —— 188	
16.4.2	Function of LSCM —— 189	
Recommended readings —— 193		
Appendi	x 1 Commonly used buffer in histochemistry —— 195	
Appendi	x 2 Histochemistry and immunohistochemistry experiments —— 199	

Index —— 225