

Table of Contents

Preface	xv
Editorial Comments	xvii
About the Authors	xix
Section 1. Routine Tube Methods	1
1-A. ABO Grouping and RhD Typing	2
1-B. Testing for Unexpected Antibodies: LISS Additive Procedure	7
1-C. Crossmatching by LISS Antiglobulin	11
1-D. Weak D Testing Procedure	15
Table 1-D-1. Interpretation Recommendations for Direct and Indirect Tests with Anti-D.	20
1-E. Crossmatching by Immediate-Spin	21
1-F. Grading and Scoring Serologic Reactions	25
Table 1-F-1. Grading Serologic Reactions	28
1-G. Intralaboratory Competency Testing: IAT Reading and Grading Procedure	29
Section 2. Antibody Detection	37
2-A. Testing by Albumin Addition/Layering	38
2-B. Testing by Albumin Antiglobulin	41
2-C. Testing by Enzyme IAT	45
2-D. Testing by LIP IAT	50
2-E. Testing by LISS Wash IAT	55
2-F. Testing by LISS-Ficin IAT	59
2-G. Testing by Polyethylene Glycol IAT	64
2-H. Testing for Cold-Reactive Antibodies by Direct Agglutination	68
2-I. Testing by Saline IAT	71
Section 3. Enzyme Techniques	75
3-A. Detecting Antibodies with the Bromelain One-Stage Method	77
3-B. Treating Red Cells with α -Chymotrypsin	80

3-C.	Detecting Antibodies with the Ficin One-Stage Method	83
3-D.	Treating Red Cells with Ficin	86
3-E.	Treating Red Cells with Neuraminidase (T-Activation).	90
3-F.	Detecting Antibodies with the Papain One-Stage Method	93
3-G.	Treating Red Cells with Papain	96
3-H.	Treating Red Cells with Pronase	100
3-I.	Treating Red Cells with Trypsin (Crude Enzyme)	104
3-J.	Treating Red Cells with Trypsin (Purified Enzyme)	108
3-K.	Treating Red Cells with ZZAP	112
Section 4.	Elution Techniques	115
4-A.	Washing Before Eluting	116
4-B.	Eluting Antibodies with Chloroform	118
4-C.	Eluting Antibodies with Chloroform/Trichloroethylene	122
4-D.	Eluting Antibodies with Citric Acid	126
4-E.	Eluting Antibodies with Cold Glycine Acid	130
4-F.	Eluting Antibodies from Stroma	134
4-G.	Eluting Antibodies with Ether	138
4-H.	Eluting Antibodies by Freezing-Thawing (Lui Method).	142
4-I.	Eluting Antibodies by Freezing-Thawing (Wiener Method)	145
4-J.	Eluting Antibodies by Heat	149
4-K.	Eluting Antibodies with Methylene Chloride	152
4-L.	Eluting Antibodies with Microwaves	156
4-M.	Eluting Antibodies from Placental Tissue	159
4-N.	Eluting Antibodies Using Ultrasound	162
4-O.	Eluting Antibodies with Xylene/D-Limonene.	165
4-P.	Dissociating IgG from Cells Using EDTA-Glycine-HCl.	169
4-Q.	Dissociating IgG from Cells Using Chloroquine Diphosphate	172
Section 5.	Cell Separation Methods	175
5-A.	Harvesting Autologous Red Cells by Direct Centrifugation	176
5-B.	Harvesting Autologous Red Cells Using Phthalate Esters.	179
5-C.	Cell Separation Using Percoll Procedure.	183

Table of Contents

5-D.	Harvesting Autologous Red Cells in Hemoglobin S or Sickle Cell Disease	186
5-E.	Separating Mixed-Cell Populations with Antibodies	189
5-F.	Mononuclear Cell Separation Procedure	192
Section 6. Alternative Serologic Methods for Antibody Detection		195
6-A.	Gel: Preparing Buffered Gel Columns	197
6-B.	Gel: Preparing Anti-IgG Columns	201
6-C.	Gel: Detecting Antibodies Using Buffered Gel Columns	205
6-D.	Gel: Detecting Antibodies Using Anti-IgG Columns	208
6-E.	Gel: Adapting Prefilled Neutral Buffer Columns for Use with Murine IgG Antibodies	211
6-F.	Gel: Adapting Prefilled Anti-IgG Columns for Use in a Modified PEG-IAT	215
6-G.	Microplate: ABO Grouping and RhD Typing Procedure	219
6-H.	Microplate: Antibody Detection Using LISS Procedure	225
6-I.	Microplate: LIP Technique Procedure	229
6-J.	Capillary: Saline Agglutination Procedure	232
6-K.	Capillary: Albumin One-Layer Procedure	235
6-L.	Capillary: Albumin Two-Layer Procedure	238
6-M.	Capillary: Papain One-Stage Procedure	241
6-N.	Capillary: Ficin One-Stage Procedure	244
6-O.	Capillary: IAT on Precoated Red Cells Procedure	247
6-P.	Capillary: Precoating for IAT Procedure	250
	Appendix 6-1. Sephadex Gel Beads: Background Information	253
	Appendix 6-2. Agglutination Reactions in Gel Columns	254
	Appendix 6-3. Microplate Tests: General Considerations	255
	Appendix 6-4. Capillary Methods: General Considerations	257
Section 7. Reagent Red Cells (Non-Enzyme-Treated)		259
7-A.	Preparing C3b/C4b-Coated Red Cells	261
7-B.	Preparing C3b/C4b-Coated Red Cells with Table Sugar	264
7-C.	Preparing IgM/C3b-Coated Red Cells	267
7-D.	Preparing C3b-Coated Red Cells: Fruitstone Method	271
7-E.	Preparing C4b-Coated Red Cells	274
7-F.	Preparing IgA- and IgM-Coated Red Cells	277

7-G.	Preparing IgG-Coated Red Cells	281
7-H.	Treating Red Cells: AET Procedure	285
7-I.	Treating Red Cells: 0.2 M DTT Procedure	287
7-J.	Inactivating S Antigen: Sodium Hypochlorite Treatment	289
7-K.	Freezing Red Cells: Glycerol Preservation and Recovery	291
7-L.	Freezing Red Cells: Liquid-Nitrogen Preservation and Recovery	294
Section 8.	Antibody Identification	299
8-A.	Identification of Single or Multiple Antibodies	300
	Figure 8-A-1. Considerations in investigating unexpected antibodies	304
	Figure 8-A-2. Considerations in investigating blood samples with a positive autocontrol	305
	Table 8-A-1. Serologic Characteristics of Some Alloantibodies to Red Cell Antigens	306
8-B.	Management of Samples and Patients with Cold Agglutinins	309
8-C.	Evaluation of Panel Results: Crossing Out	313
	Figure 8-C-1. Antigens crossed out on each individual panel cell	315
	Figure 8-C-2. Antigens crossed out on the top of the antigen profile	315
8-D.	Selected Cells Process	316
8-E.	Selection of Antigen-Negative Blood for Transfusion	319
	Table 8-E-1. Selection of Antigen-Negative Blood for Transfusion: Some Examples of Commonly Encountered Antibodies	323
8-F.	Determination of Probability Levels	324
	Table 8-F-1. Probability Values.	328
8-G.	Acidifying Sera	329
8-H.	Using a Combined Adsorption/Elution Procedure	331
8-I.	Detecting Trypsin-Resistant N _{VG} Receptors	335
8-J.	Using Soluble Blood Group Substances in an Inhibition Procedure	338
	Table 8-J-1. Antibody Inhibition by Blood Group Substances	341
8-K.	Inhibiting Rouleaux with DIDS	342
8-L.	Identifying Anti-N _{form}	345
8-M.	Detecting Complement-Fixing Antibodies by a Two-Stage EDTA-IAT	348
	Appendix 8-1. Evaluation of Initial Antibody Identification Panel	351
	Appendix 8-2. Approaches to Identify/Confirm Antibody Specificity	352

Table of Contents

Section 9. Investigating Antibodies to High-Prevalence Antigens	353
Table 9-1. Effect of Papain/Ficin and Thiol Reagents on Reactions of Antibodies to High-Prevalence Red Cell Antigens	357
Table 9-2. Effects of Enzymes and Chemicals on Reactions of Antibodies to High-Prevalence Red Cell Antigens	358
Figure 9-1. Investigation of antibodies to high-prevalence antigens	359
9-A. Inhibiting Antibodies with Pooled Plasma or Serum	360
9-B. Identifying Anti-Ch/Rg: Rapid Procedure	363
9-C. Recognizing Antibodies to HLA Determinants	366
9-D. Using Allogeneic C4 for Adsorption of Anti-Ch/Rg	369
9-E. Using 2 M Urea for the Screening/Confirmation of Jk(a-b-) Red Cells	372
Section 10. Prenatal and Perinatal Testing	375
10-A. Tests During Pregnancy	381
10-B. Rh Immune Globulin Administration	384
Figure 10-B-1. Flow diagram for Rh Immune Globulin administration	388
10-C. Evaluation of Hemolytic Disease of the Fetus and Newborn at Delivery	389
10-D. Prenatal Antibody Titration Procedure	392
10-E. Differentiating Anti-G from Anti-C + Anti-D by Adsorption/ Elution	397
10-F. Screening for Fetal RhD-Positive Red Cells in Maternal RhD-Negative Samples	401
10-G. Quantifying Anti-D with the Enzyme-Linked Antiglobulin Test	405
10-H. Screening for Large Fetomaternal Hemorrhage (Modified Kleihauer-Betke Acid-Elution Test)	410
10-I. Quantifying Fetomaternal Hemorrhage with the Enzyme-Linked Antiglobulin Test	414
10-J. Analyzing Amniotic Fluid by Spectrophotometry	419
10-K. Treating Cord Blood Contaminated with Wharton's Jelly	423
Section 11. Investigating Samples Containing Autoantibodies	425
Table 11-1. Specificity of Cold-Reactive Autoantibodies: Comparison of Reactions with Untreated O, I+ Red Cells	429
11-A. Investigation of Cases of Immune Hemolysis and/or a Positive DAT	430
11-B. Antiglobulin Testing: Direct Tests with Anti-IgG and Anti-C3 (Test Tube Method)	436

11-C.	Antiglobulin Testing: Direct Tests with Anti-IgM and Anti-IgA (Test Tube Method)	440
11-D.	Antiglobulin Testing: Direct Tests with Anti-IgM and Anti-IgA (Column Agglutination Test)	443
11-E.	Antiglobulin Testing: Direct Tests with Polybrene	446
11-F.	Antiglobulin Testing: Enzyme-Linked DAT	450
11-G.	Dispersing Autoagglutination	454
11-H.	Testing the Thermal Amplitude of a Cold-Reactive Autoantibody.	456
11-I.	Diagnostic Testing of a Cold-Reactive Autoantibody by Titration (Overnight Method)	459
11-J.	Testing of a Cold-Reactive Autoantibody by Titration.	462
11-K.	Determining Specificity of a Cold-Reactive Autoantibody by Titration	465
11-L.	Adsorbing Cold-Reactive Autoantibodies with Autologous Red Cells	468
11-M.	Adsorbing Cold Autoantibodies with Heterologous (Rabbit) Red Cells	471
11-N.	Testing for PCH Using the Donath-Landsteiner Test	475
11-O.	Determining the Relative RH Specificity of Warm-Reactive Autoantibodies by Titration	478
11-P.	Adsorbing Warm-Reactive Autoantibodies with Enzyme-Treated Autologous Red Cells	481
11-Q.	Adsorbing Autoantibodies with ZZAP-Treated Autologous Red Cells	485
11-R.	Adsorbing Autoantibodies with ZZAP-Treated Autologous Red Cells (Micromethod)	489
11-S.	Adsorbing Warm-Reactive Autoantibodies with Allogeneic Red Cells	493
11-T.	Evaluating Alloadsorbed Serum Results: Crossing Out	497
11-U.	Prewarming Indirect Antiglobulin Testing	503
Section 12.	Investigating Drug-Induced Hemolysis	507
	Table 12-1. Drugs Reported to Cause Immune Hemolysis and a Positive DAT.	510
12-A.	Dissolving Drugs in Solution	515
12-B.	Preparing Urine for Detecting Metabolite-Dependent Antibodies.	522
12-C.	Detecting Drug-Dependent Antibodies Reacting with Drug-Treated Red Cells	525
12-D.	Detecting Antibodies to Semisynthetic Penicillins	531
12-E.	Detecting Cephalothin-Dependent Antibodies	535
12-F.	Detecting Drug-Dependent Antibodies Reacting in the Presence of Drug.	538
12-G.	Detecting Metabolite-Dependent Antibodies Reacting in the Presence of Drug	545

Table of Contents

Section 13. Investigating ABO Grouping Problems	551
Table 13-1. Causes of ABO Grouping Discrepancies	558
Table 13-2. Some Anomalous or Uncommon ABO Grouping Results	559
Figure 13-1. Flow chart for the investigation of ABO discrepancies.	560
13-A. Investigation of Mixed-Field Reactions	561
13-B. Investigation of Nonconcordant ABO Grouping Reactions	563
13-C. Evaluating an ABO Discrepancy	567
13-D. Using Protease-Treated Red Cells for ABO Grouping Resolution	571
13-E. Confirming Weak A or B Antigen Expression by Adsorption and Heat Elution	573
13-F. Confirming Weak A or B Antigen Expression by Adsorption and Freezing-Thawing (Lui) Elution	577
13-G. Detecting Minor Red Cell Populations and ABO Mixtures	581
13-H. Determining ABH Secretor Status	584
13-I. Determining H Antigen Expression	587
13-J. Separating A:O Mixtures	590
13-K. Detecting A and B Transferases by Conversion of Group O Red Cells	594
13-L. Prewarming ABO Reverse Grouping	597
13-M. Selecting Plasma Containing High-Titer Anti-A and/or Anti-B	600
Section 14. Investigating Red Cell Polyagglutination	603
Table 14-1. Reactions of Polyagglutinable Red Cells with Lectins.	604
14-A. Detecting Polyagglutination with AB and Cord Blood Serum.	605
14-B. Preparing Crude Extracts of Lectins	608
14-C. Preparing Crude Extracts of Salvia Lectins	612
14-D. Preparing Solutions of Purified Lectins	616
14-E. Aggregating Red Cells with Polybrene.	619
14-F. Acetylating Red Cells	622
14-G. Inhibiting Acquired-B Reactivity	626
14-H. Testing Red Cells with Acidified Anti-B.	629
Section 15. Miscellaneous Methods.	631
15-A. Testing for PNH: Acid Hemolysis (Ham's) Test.	632
Table 15-A-1. Acid Hemolysis Test Procedure	635
15-B. Testing for HEMPAS: Modified Acid Hemolysis (Ham's) Test	636

Judd's Methods in Immunohematology

15-C.	Converting IgG Antibodies to Direct Agglutinins.	639
15-D.	Assessing Red Cell Survival Using ⁵¹ Cr-Labeled Red Cells	642
15-E.	Differentiating IgM from IgG Antibodies Using 0.01 M DTT	647
	Table 15-E-1. Interpretation of 2-ME/DTT Studies to Determine Immunoglobulin Class of Antibody	650
15-F.	Differentiating IgM from IgG Antibodies Using 2-ME	651
15-G.	Performing Dosage Studies Using the Enzyme-Linked Antiglobulin Test	655
15-H.	Predicting Clinical Significance Using the Monocyte Monolayer Assay	660
15-I.	Screening for PNH: Sucrose Hemolysis Test.	665
15-J.	Converting Plasma to Serum	668
15-K.	Isolating Genomic DNA from Peripheral Blood	671
15-L.	Amplifying Genomic DNA Using a Hot Start Taq PCR.	674
15-M.	Preparing and Running an Agarose Gel for Analysis of PCR Products	679
Appendices.	683
	Appendix A. Reagent Preparation and Storage	684
	Appendix B. Reagent Preparation Documentation Form.	703
Index	705