CONTENTS

1. Introduction **1**
Usha, Gyanendra Kumar Sonkar, Sangeeta Singh
- Common Investigations for Immunological Disorders *1*
- Laboratory Tests for Antigen and Antibody Detection *3*
- Radioimmunoassay (RIA) *5*
- Enzyme-Linked Immunosorbent Assay (ELISA) *6*
- Flow Cytometry *8*
- Immunoperoxidase Test *11*
- Molecular Genetic Technique in Immunopathology *12*
- Polymerase Chain Reaction (PCR) *16*
- DNA Chip Technology *20*

2. Tests for Cell Mediated Immunity (CMI) **21**
Usha, Gyanendra Kumar Sonkar
- Test for Lymphocyte Activation and Proliferation *22*
- Cytokines *22*
- Diagnosis of Tuberculosis in High-risk Area by DTH Test *25*
- Delayed Type Hypersensitivity (DTH) Response in Leprosy (Lepromin Test) *26*
- Application of DTH Response in Leishmania Infection *27*
- Contact Sensitization with 2:4 Dinitrochlorobenzene (DNCB) to Test CMI *27*

3. Lymphocytes Typing by Flow Cytometry **29**
Shyam Sundar, Madhukar Rai, Radheshyam Maurya, Krishna Das Manandhar
- Objectives *29*
- Method *29*
- Materials Required *29*
- Standard Operating Procedure *30*
- Antibody Panels *31*
- Interpretations of CMI Tests *31*

4. Diagnosis of Kala-azar by PCR Technique **33**
Shyam Sundar, Madhukar Rai, Radheshyam Maurya, Krishna Das Manandhar
- Objective *33*
- Method *33*
- Materials Required *33*
- Polymerase Chain Reaction (PCR) *33*
- Standard Operating Procedure *34*

5. Test for Myeloma Profile and Qualitative Assessment of Serum Proteins **37**
Usha
- Paper Electrophoresis of Serum and Urine *37*
- In Plasma Cell Dyscrasia *39*
- Appendix *39*
- Tests for Bence Jones Proteins in Urine *40*

6. Agarose Gel Electrophoresis for Qualitative Assessment of Serum Proteins **43**
Usha, Gyanendra Kumar Sonkar, Sangeeta Singh, Suman Singh
- Principle *43*
- Material *43*
- Method *44*
- Appendix *45*

7. Cellulose Acetate Electrophoresis **48**
Usha, Gyanendra Kumar Sonkar, Sangeeta Singh
- Principle *48*
- Method *48*
- Solutions *49*
- Applications *49*
- Points to be Noted *50*
- Advantages *50*

8. Agar Gel Immunoelectrophoresis for Typing of Myeloma **51**
RM Gupta
- Principle *51*
- Equipment *51*
- Reagents *51*
- Microslides *52*
- Procedure *52*
- Preparation of Agar Gel Slides *52*

9. Micro Agar Gel Double Diffusion Technique **54**
RM Gupta
- Principle *54*
- Reagents *54*
- Preparation of Agar Plate *54*

10. Counter Current Immunoelectrophoresis **56**
RM Gupta, Usha
- Principle *56*
- Laboratory Procedure *56*

11. Single Radial Immunodiffusion and Immunoturbidometric Methods for the Estimation of Immunoglobulins **57**
RM Gupta, Usha
- Single Radial Immunodiffusion (SRID) *61*
- Immunoturbidometric Method for Estimation of Immunoglobulins *63*
- Comparison of SRID and Turbidometry Test for Estimation of Immunoglobulins and Complements *65*

12. Estimation of Complement C3, C4 and Hemolytic Assay for Serum Complement **68**
Usha
- Estimation of Complement C3 and C4 *68*
- Immunoturbidometric Assay for Determination of Complements C3 and C4 *69*

- Hemolytic Assays for Complement Estimation *70*
- Appendix *72*

13. Demonstration of Immune Complexes and Complement by Indirect Immunoperoxidase Method **75**
Bharat Jasani, Keith-Miller
- Principle of the Test *75*
- Procedure *76*
- Interpretation *76*
- Disadvantage *76*
- Additional Notes *76*

14. Test for Autoimmune Diseases **77**
Usha
- Demonstration of Lupus Erythematosus (LE) Cells in Peripheral Blood *78*
- Antinuclear Antibody (ANA) by Indirect Immunofluorescent Test *80*
- Indirect Fluorescent Test for Autoimmune Chronic Hepatitis, Primary Biliary Cirrhosis, Atrophic Gastritis and Good Pasteur's Syndrome *83*
- Indirect Immunofluorescent Test for Autoimmune Hepatic Disease *84*
- Antineutrophil Cytoplasmic Antibody (ANCA) Test *89*
- Detection of Antikeratin Antibody, Antiendomysial Antibody and Antidesmosomal (Desmoglein) Antibody by Indirect Fluorescent Method *91*

15. Test for Autoantibodies by ELISA Technique **96**
Usha
- Enzyme-linked Immunosorbent Assay for IgM Rheumatoid Factor (RF) *96*

16. Acid Fuchsin Orange G in Kidney Biopsy for Demonstration of Immune Complexes **98**
HU Zollinger, MJ Mihatsch
- Acid Fuchsin Orange G (AFOG) Staining *98*
- Appendix *99*

17. Detection of Tumor Markers in Paraffin-embedded Sections by Immunohistochemistry **102**
Bharat Jasani, Usha, Amita Chaubey
- Indirect Immunoperoxidase Staining for C-erb B2 Antigen in Tissue Section *102*
- P53 Antigen Detection in Paraffin-embedded Sections *104*
- Appendix for Immunohistochemistry *105*

18. Detection of Circulating Immune Complexes (CIC) in the Serum **107**
RM Gupta, Usha
- Principle *107*
- Method *107*
- PEG Index: Multiply Reading of Absorbance with 100 *107*
- Threshold Value *108*
- Appendix *108*

19. Demonstration of Immune Complexes, Antibodies and Complements in Renal Biopsy by Immunofluorescence **109**
Usha
- Direct Immunofluorescent Test *109*
- Indirect Immunofluorescent Test *110*

20. HLA Typing and Cross Match Test in Solid Organ Transplantation **113**
Gyanendra Kumar Sonkar, Sangeeta Singh, Usha
- Major Histocompatibility Complex *113*
- Inheritance of HLA Genes *117*
- Typing of HLA Class I (A, B, C) Antigens *117*
- Typing of HLA Class II (DR, DQ) Antigens *119*
- Cross Match Test for Renal Transplantation *120*
- Determination of HLA Class II by Immunobead Method *123*
- Appendix *124*

21. HLA B27 Typing by PCR Method **126**
Usha, Gyanendra Kumar Sonkar, Pramod Kumar Verma, Sangeeta Singh, Suman Singh
- Principle *126*
- Method of DNA Extraction *127*
- Gel Electrophoresis of the PCR Products *128*
- Appendix *129*
- Significance of Human Leukocyte Antigen (HLA) Typing *130*
- Human Leukocyte Antigens in Normal Population *131*

22. Fluorescence In Situ Hybridization: Methods and Applications **135**
Nuzhat Husain, Priyanka Soni
- Probes *137*
- FISH on Paraffin-embedded Tissue Sections *138*
- FISH on Blood Smear *140*
- Interpreting FISH Signals *141*
- Tips and Troubleshooting *143*
- Applications *144*

23. Detection of Cytokines (IL-6, IL-8, TNF-α) **148**
Tripti Sharma, Gyanendra Kumar Sonkar, Sangeeta Singh, Usha
- Detection of Il-6 by ELISA Test *148*
- Appendix for ELISA Test *149*

24. Western Blot or Tests for Detection of Antibody to Extractable Nuclear Antigen **151**
Amita Agrawal
- Separation of Extractable Nuclear Antigens from Rabbit Thymus *151*
- Preparation of SDS-PAGE Electrophoresis *152*
- Prepare Reagents in Double Distilled Water *152*
- Staining Procedures *153*
- Appendix *154*

25. Detection of Antithyroglobulin Antibodies by Hemagglutination Method 157
Amita Agrawal
- Hypothyroidism *157*
- Detection of Antithyroglobulin Antibodies (ATG Ab) *158*
- Indirect Fluorescent Test for Antithyroglobulin and Antithyroid Peroxidase Antibody *159*
- Gelatin Agglutination Method for Antithyroid Microsomal Antibodies *160*

26. Distillation and Demineralization of Water 162
HD Khanna, Shruti Khanna
- Water Distillation Apparatus *162*
- Deionizers (Demineralizer) *163*

27. Raising of Polyclonal Anti-human Anti-IgG in Rabbit 167
Usha
- Method *167*
- Bleeding of Rabbits *167*
- Points to be Noted *168*

Index *169*