

Contents

Contributors	xi
Acknowledgments	xiii
Preface	xv

Basic Methods

1. Basic Flow Cytometry Instrumentation	3
<i>Charles L. Goolsby and Cathy James</i>	
Instrumentation	3
Overview	3
Delivery of Sample	4
Light Source and Signal Detection	5
Data Analysis	10
Gating	13
Color Compensation	15
Conclusion	20
2. Fixation for In Situ Molecular Analysis	23
<i>Bruce K. Patterson and Daniel K. Jiyamapa</i>	
Introduction	23
Types of Fixatives	24
Cells	27
Tissue	29
3. A Method for Reduction of Green Wavelength Autofluorescence Emission in Cellular Preparations	35
<i>Victoria L. Mosiman</i>	
Protocols—Flow Cytometry	39
Trypan Blue with Surface Staining	39
Surface Stain Plus In Situ Hybridization	40
Surface Label Plus In Situ PCR or RT-PCR	40

4. Gene Quantification: Choosing the Target	45
<i>Bruce K. Patterson</i>	
Introduction	45
Fresh Cells and Tissue	45
Quantification of Gene Expression Prior to In Situ Analysis	50
5. Detection and Quantification of Cytokine-Producing Cells by Immunostaining	55
<i>Ulf Andersson, Mark J. Litton, Tom E. Fehniger, Ann-Kristin Ulfgren, Jan Andersson</i>	
Introduction	55
Materials	58
Methods	61
Procedure 1: Cytokine Detection of Individual Cells	
Smeared on Slides Using Immunostaining	61
Quantification of Individual Cytokine-Producing Cells by Automated Computerized Image Analysis (ACIA)	64
Assessment of Cell Size, Intensity of Signal, and Enumeration of Individual Cytokine-Producing Cells	65
Procedure 2: Immunofluorescent Staining of Cytokine-Producing Cells in Suspension	71
Procedure 3. Cytokine Detection in Tissue Using Immunohistochemistry	73
Troubleshooting	75
6. In Situ Hybridization Using the bDNA Technology	81
<i>Vincent P. Antao, Audrey N. Player, and Janice A. Kolberg</i>	
Introduction	81
Materials and Methods	83
Reagents	84
Preparation of Cytospin Slides	84
Pretreatment of Slides Prior to Hybridization	85
Hybridization and Signal Development	85
Results and Discussion	87
Conclusions	92
Acknowledgments	92

Specific Applications

7. Kaposi's Sarcoma-Associated Human Herpes Virus Eight: Localization of Viral Gene Expression and Viral Latency	97
<i>Scott J. Brodie, John N. Krieger, Catherine Diamond, Kurt Diem, and Lawrence Corey</i>	

Introduction	97
Materials and Methods	98
Clinical Samples	98
Solution-Based PCR and Serologic Assays.....	98
In Situ Hybridization	100
In Situ Polymerase Chain Reaction.....	101
Results	101
Discussion.....	102
8. Pathogenetic Mechanisms of Animal Orbiviruses That Cause Disease at Low Copy Number	107
<i>Scott J. Brodie, Patricia M. O'Hearn, Kurt Diem, and David Muthui</i>	
Introduction	107
Materials and Methods	108
Experimental Design.....	108
Clinical Samples	108
Virus Isolation	109
Immunochemistry	109
Nested Reverse Transcription Polymerase Chain Reaction	110
In Situ Hybridization	111
Reverse Transcription In Situ Polymerase Chain Reaction.....	112
Results	113
Clinicopathologic Features	113
Isolation and Typing of Viruses	113
Tissue Distribution and Cellular Localization of Viral Proteins and Nucleic Acids.....	114
Discussion.....	117
9. Localization of Hepatitis C Virus Using Reverse Transcriptase Fluorescence In Situ 5' Nuclease Assay.....	123
<i>Bruce K. Patterson</i>	
Introduction	123
Determining the Localization Method	123
Detection of Gene Expression in Specific Cell Types.....	126
10. Simultaneous Quantification and Localization of HIV gag-pol Expression Using Ultrasensitive Fluorescence In Situ Hybridization.....	135
<i>Bruce K. Patterson</i>	
11. Quantification and Localization of Gene Expression: Future Directions	143
<i>Bruce K. Patterson</i>	
Index	147