

Contents

Preface *xiii*

Acknowledgments *xvii*

Nomenclature *xix*

1 Introduction 1

Mammalian Cells: Modern Workhorses 1

Products from Cells 1

Cells as Therapeutic Agents 2

Biomarkers for Health or Disease 2

In Vitro Models 3

Bridging the Gap 3

The Preservation Toolkit 5

Hypothermic Storage 5

Cryopreservation 6

Vitrification 7

Dry State Storage 8

Fit-for-Purpose 8

One Size Does Not Fit All 9

The Process is the Product 9

Reproducibility 12

Safety 12

Dispelling the Myth of the Cold Black Box 12

References 13

2 Pre-freeze Processing and Characterization 15

Pre-freeze Processing 15

Digestion of Cells from Intact Tissue 15

Hypothermic Storage 16

Selection of Subpopulations 17

Activation or Stimulation 18

Genetic Modification 18

Culture 19

Pre-freeze Process Monitoring 19

Pre-freeze Characterization	20
Identity	20
Genetic Stability	21
Enumeration	21
Purity	22
Adventitious Agents	22
Microbial Testing of Cell Therapy Products	23
Special Considerations for the Characterization of Cell Therapies	24
Annotation of Pre-freeze Processing	24
Scientific Principles	25
Putting Principles into Action	25
References	26

3 Formulation and Introduction of Cryopreservation Solutions	29
Importance of Cryoprotective Agents	29
Mechanisms of Cryoprotection	31
Formulating a Cryopreservation Solution	31
Formulation of a Vitrification Solution	33
Characterization and Quality Control for Cryoprotective Solutions	34
Toxicity of CPAs	35
Osmotic Toxicity	35
Biochemical Toxicity	36
Developing a Protocol for Introducing CPA Solutions	37
The Basic Experiment	37
Introduction of Vitrification Solutions	38
Cell Concentration	39
Removal of CPA Solution	40
Safety Considerations for Cryopreservation Solutions	40
Cryopreservation Containers	41
Overwraps	42
Labeling	43
Sample Annotation	44
Scientific Principles	44
Putting Principles into Practice	44
References	44

4 Freezing Protocols	47
Importance of Cooling Rate	47
Controlled-rate Freezing	48
Controlled Cooling-rate Protocols	49
Segment 1: Initial Hold Period	49
Segment 2: Cooling	50
Uncontrolled Nucleation	53

Manual Nucleation	54
Automatic Nucleation	54
Verifying Segment 2 (Including S2a)	55
“Delayed” Latent Heat	55
Segment 3	56
Verifying Segment 3	56
Other Types of Controlled-rate Protocols	57
Passive Freezing	57
Transfer to Storage	59
Vitrification	60
Independent Temperature Measurement	60
Scientific Principles	61
Putting Principles into Practice	62
References	62
5 Storage and Shipping of Frozen Cells	65
Scientific Basis for Selection of a Storage Temperature	65
Additional Considerations for Vitrified Samples	67
Standards, Guidelines, and Best Practices	67
Facilities	68
Storage Equipment and Environment	69
Mapping Storage Devices and Setting Alarm Limits	70
Monitoring Systems	71
Safety	71
Inventory Management System	72
Stability in Storage	72
Temperature Fluctuations	73
Influence of Background Ionizing Radiation on Stability	
in Storage	74
Shelf-Life of Samples in Storage	75
Fit-for-Purpose Storage Practices	75
Risk Mitigation in Long-Term Storage	76
Shipping or Transport of Cells	76
General Shipping Considerations	77
Liquid Nitrogen Dry Shippers	78
Temperature Mapping of a Shipper	79
Packaging of Samples Being Shipped	79
Monitoring of Shipments	79
Responsibilities	79
Sample Annotation	80
Scientific Principles	80
Putting Principles into Practice	81
References	81

6 Thawing and Post-Thaw Processing	85
Thawing Equipment	86
Transporting Samples Prior to Thawing	87
Estimating Your Thawing Rate	87
Thawing and Infusion of Cell Therapy Products	89
Safety Considerations for Thawing	90
Post-Thaw Processing	90
Post-Thaw Washing	90
Dilution	91
Infusion of Cells Immediately Post-Thaw	91
Removal of Vitrification Solutions	92
Wash Solutions	92
Scientific Principles	94
Putting Principles into Practice	94
References	94
7 Post-Thaw Assessment	97
Common Measures Used in Post-Thaw Assessment	98
Physical Integrity	98
Metabolic Activity	99
Mechanical Activity	100
Mitotic Activity	101
Differentiation Potential	102
Transplantation Potential	103
Strategies to Improve the Accuracy and Reproducibility of Post-Thaw Assessment	103
Eliminate Measurement Bias	103
Compensating for Post-Thaw Apoptosis	105
Post-Thaw Assessment Using a Single Measure	106
Optical Methods of Post-Thaw Assessment	106
Release Criteria	107
Scientific Principles	107
Putting Principles into Practice	107
References	108
8 Algorithm-Driven Protocol Optimization	111
Small Cell Number/High Throughput Approach	113
Validating Operation of the Algorithm	114
Flexibility	115
Practical Notes	115
Modeling in Cryobiology	115
References	116

Protocols

Introduction 117

Protocol Contributors 118

Cryopreservation of Endothelial Cells in Suspension 119

Principle 119

Equipment and Supplies 119

Equipment 119

Supplies 120

Safety 120

Procedure 121

Cell Preparation 121

Preparation of Cryoprotectant Solution 121

Using Powdered HES 122

Using Pentastarch Solution 122

Cryoprotectant Addition 122

Freezing 122

Controlled-rate Freezing with a

Methanol Bath 122

Alternative Freezing Procedure 123

Thawing 123

Expected Results 123

References 123

Cryopreservation of Peripheral Blood Mononuclear Cells from Whole Blood 125

Principle 125

Protocol 1: Isolation of PBMCs Directly over

Ficoll-Hypaque 125

Equipment 125

Materials 126

Reagents 126

Procedure 126

Protocol 2: Isolation of PBMCs Using SepMate[®] -127

Equipment 127

Materials 128

Reagents 128

Procedure 128

Appendix A Human Serum AB Freezing Media 129

Materials 129

Equipment 130

Reagents 130

Procedure 130

Cryopreservation of Human Adipose Stem Cells 131

Principle 131

Equipment and Supplies 131

Reagents and Media 132

Procedure 133

Isolation of Human ASCs from Lipoaspirate 133

Magnetic Cell Sorting (Optional) 135

Cryopreservation 135

Controlled-rate Freezing of Human ASCs 135

Thawing Human ASCs 137

Notes 138

Reference 139

Cryopreservation of Red Blood Cells 141

Method I: High Glycerol/Slow Cooling Technique

(Meryman and Hornblower 1972) 141

Preparation of the RBC Concentrate 141

Addition of the Cryoprotective Solution 141

Cooling 142

Rewarming 142

Removal of the Cryoprotectant and Debris 142

Method II: A Low Glycerol/Rapid Cooling Technique

(Rowe, Eyster, and Kellner 1968) 143

Method III: Hydroxyethylstarch/Rapid Cooling Technique

(Sputtek 2007) 144

References 146

Cryopreservation of Oocytes by Slow Freezing 147

Principle 147

Specimen Requirements 147

Equipment and Supplies Needed 147

Equipment 147

Supplies 148

Procedure 148

Safety 152

Calculations 152

Reporting Results 152

Procedure Notes 153

Limitations of Procedure 153

Oocyte Vitrification and Warming 155

Principle 155

Equipment and Supplies 155

Equipment	155
Supplies	155
Procedure	156
Quality Control	160
Safety	161

Transportation of Hematopoietic Progenitor Cells and Other Cellular Products 163

Principle/Rationale	163
Specimen	163
Equipment/Reagents	163
Quality Control	164
Procedure	164
Additional Information	165
Further Reading	165

Cryopreservation of Hematopoietic Progenitor Cells 167

Principle/Rationale	167
Protocol/Processing Schema	168
Specimen	168
Equipment/Reagents	168
Quality Control	169
Procedure	169
Appendix A Alternate Cryopreservation	
Harness Set-2 or 4 Bags	173
Further Reading	173

Thawing of Hematopoietic Progenitor Cells 175

Principle/Rationale	175
Equipment/Reagents	175
Quality Control	176
Procedure	176
Further Reading	177

Processing and Cryopreservation of T-Cells 179

Principle/Rationale	179
Protocol/Processing Schema: N/A	179
Specimens	179
Equipment/Reagents	179
Quality Control	180
Procedure	180
Further Reading	182

Thawing and Reinfusion of Cryopreserved T-Cells 183

Principle/Rationale 183

Protocol/Processing Schema 183

Specimen 184

Equipment/Reagents 184

Quality Control 184

Procedure 184

Further Reading 187

Index 189