

## Chapter 8.2

# Cryopreservation of Lymphocytes in Bulk

DOUGLAS M. STRONG

### Introduction

Since the early discovery of the cryoprotective properties of glycerol, a great deal of investigation has gone into the determination of the mechanisms of freezing injury (Polge et al., 1949). Very briefly, cells that are cooled too slowly, to below freezing temperatures, are damaged by the resulting increase in salt concentration and cellular shrinkage which occurs as water is removed during the formation of extracellular ice (Lovelock, 1953). Conversely, if cooling is too rapid, a new mechanism is invoked in which shrinkage no longer occurs but the cell is damaged by the formation of intracellular ice, either during freezing or upon thawing (Mazur et al., 1969). Cryoprotectants, such as  $\text{Me}_2\text{SO}$ , reduce the amount of ice present during freezing and reduce solute concentration thus reducing ionic stress (Farrant et al., 1974; Lovelock, 1953; Mazur et al., 1969; Polge et al., 1949). However, these compounds can themselves cause osmotic injury since they are hypertonic and can cause damage during their addition or removal (Strong, 1976). Optimum cooling rates vary from cell type to cell type depending on differences in membrane permeability and intracellular water, which is removed during the dehydration phase of slow cooling and extracellular ice formation. In general, the larger the cell volume, the slower the rate of cooling to allow equilibration of intra- and extra-cellular water during freezing.

Although the primary use of cryopreserved lymphocytes in the histocompatibility laboratory may be as reagents in lymphocytotoxicity assays, where the preservation requirements are less rigid, the use of frozen thawed cells in functional assays or in assays for determination of other cell surface markers, may require closer attention to protocol. Several laboratories have reported that, under certain conditions, selection of lymphoid subsets may occur following freezing and thawing (Farrant et al., 1972; Knight et al., 1972). Furthermore, lymphoid clones and B- or T-LCL have different optimum cooling rates (Strong et al., 1985). Although sophisticated controlled rate freezing devices are not absolutely required for lymphocyte cryopreservation, such equipment usually increases reproducibility and improves recovery (Dubey et al., 1986; Ichino and Ishikawa, 1988). In addition, the handling of cells prior to and following freezing and thawing is at least as important as the freezing itself.

### Abbreviations

$\text{Me}_2\text{SO}$ : Dimethylsulfoxide  
DNase: Deoxyribonuclease

# Ashi Laboratory Manual

**Mary S. Leffell, Albert D.  
Donnenberg, Noel R. Rose**



## **Ashi Laboratory Manual:**

*ASHI Laboratory Procedure Manual* American Society for Histocompatibility and Immunogenetics, Amy B. Hahn, Geoffrey A. Land, Rosemarie M. Strothman, 2000      Manual of Molecular and Clinical Laboratory Immunology Barbara Detrick, John L. Schmitz, Robert G. Hamilton, 2020-07-16 THE authoritative guide for clinical laboratory immunology For over 40 years the Manual of Molecular and Clinical Laboratory Immunology has served as the premier guide for the clinical immunology laboratory From basic serology testing to the present wide range of molecular analyses the Manual has reflected the exponential growth in the field of immunology over the past decades This eighth edition reflects the latest advances and developments in the diagnosis and treatment of patients with infectious and immune mediated disorders The Manual features detailed descriptions of general and specific methodologies placing special focus on the interpretation of laboratory findings and covers the immunology of infectious diseases including specific pathogens as well as the full range of autoimmune and immunodeficiency diseases cancer and transplantation Written to guide the laboratory director the Manual will also appeal to other laboratory scientists especially those working in clinical immunology laboratories and pathologists It is also a useful reference for physicians mid level providers medical students and allied health students with an interest in the role that immunology plays in the clinical laboratory      Manual of Molecular and Clinical Lab Immunology Barbara Detrick, Robert G. Hamilton, James D. Folds, 2006-03-10 Introduces new material that reflects the significant advances and developments in the field of clinical laboratory immunology Provides a comprehensive and practical approach to the procedures underlying clinical immunology testing Emphasizes molecular techniques used in the field of laboratory immunology Updates existing chapters and adds significant new material detailing molecular techniques used in the field Presents guidelines for selecting the best procedures for specific situations and discusses alternative procedures Covers aspects of immunology related disciplines such as allergy autoimmune diseases cancers and transplantation immunology      **Manual of Molecular and Clinical Laboratory Immunology** John L. Schmitz, Barbara Detrick, Maurice R. O'Gorman, 2024-12-24 THE authoritative guide for clinical laboratory immunology For nearly 50 years the Manual of Molecular and Clinical Laboratory Immunology has been the premier resource for laboratories students and professionals involved in the clinical and technical details of diagnostic immunology testing The 9th Edition continues its tradition of providing comprehensive clinical and technical information on the latest technologies used in medical and diagnostic immunology Led by a world renowned group of authors and editors this new edition reflects substantial changes aimed at improving and updating the Manual s utility while reflecting the significant transformations that have occurred since the last edition including the revolution of gene editing and the widespread adoption of molecularly engineered cellular therapies Topical highlights include Laboratory Management three new chapters cover essential aspects of quality assurance quality improvement and quality management aligning with the increasingly stringent and demanding regulatory environment Inborn Errors of Immunity the primary immunodeficiency

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*Handbook of Human Immunology* Mary S. Leffell, Albert D. Donnenberg, Noel R. Rose, 1997-04-10 Combining basic explanations of laboratory tests with 115 tables full of reference data and applications the Handbook of Human Immunology provides practicing clinicians with a current complete guide to molecular immunology Introductory chapters overview the molecular basis of immune responses and immunological disorders focusing on the role of cell receptors accessory molecules and cytokines in these processes Emphasis is placed on immunological parameters that are clinically useful The basic principles underlying assays of the immune system are discussed and the book stresses the application and interpretation of immune tests Comprehensive coverage is given to immunoglobulins and their age dependent concentration Cellular immunology is discussed from the perspectives of lymphocyte functional parameters as well as through immunophenotyping of lymphocytes and other leukocytes Both serological and molecular diagnosis of infectious diseases are reviewed The Handbook of Human Immunology contains up to date information on exciting developments in immunogenetics covering the application of T cell receptor genes and the HLA alleles in disease associations and transplantation

**Immunohematology: Principles and Practice** Eva D. Quinley, 2020-06-15 Immunohematology Principles and Practice Third Edition an ideal text for anyone who wants to master the theory and practices of today's blood banking

Clinical Laboratory Medicine Kenneth D. McClatchey, 2002 This thoroughly updated Second Edition of Clinical Laboratory Medicine provides the most complete current and clinically oriented information in the field The text features over 70 chapters seven new to this edition including medical laboratory ethics point of care testing bone marrow transplantation and specimen testing providing comprehensive coverage of contemporary laboratory medicine Sections on molecular diagnostics cytogenetics and laboratory management plus the emphasis on interpretation and clinical significance of laboratory tests why a test or series of tests is being done and what the results mean for the patient make this a valuable resource for practicing pathologists residents fellows and laboratorians Includes over 800 illustrations 353 in full color and

270 new to this edition Includes a Self Assessment and Review book      Molecular Biology of Hematopoiesis 5 Nader G. Abraham, Shigetaka Asano, G. Brittinger, G.J.M. Maestroni, R.K. Shadduck, 2012-12-06 This volume of Molecular Biology of Hematopoiesis is dedicated to John W Adam son M D Tadimitsu Kishimoto M D Robert C Gallo M D Arthur W Nienhuis M D and Franco Mandelli M D for their contributions in developing an overall view of the state of the art knowledge in the field of hematopoiesis Richard Champlin among other renowned clinicians presented updated information on stem cells and T cell depletion for bone marrow transplant A clinical update on thrombopoietin was presented by Pamela Hunt of Amgen and by Kenneth Kaushansky Arthur Nienhuis and Katherine Turner s contribu tions to our current knowledge and advances in the fields of growth factors and gene transfer were also recognized during the 9th Symposium on Molecular Biology of Hematopoiesis in Genoa The chapters cover such diverse areas as preclinical and clinical updates on growth factors and positive and negative regulatory molecules Advances in Leukemia Mechanism and Treatment by Interferon was presented by Professor Sante Tura Readers will find presentation of exciting advances that have occurred in the area of hematopoiesis The elucidation of gene structures of key growth factor proteins such as IL 12 and IL II will lead to new insights and new approaches in understanding the regulation of hematopoiesis as well as application of new growth factors

**Histocompatibility Testing** Jeffrey L Bidwell, Cristina Navarrete, 2000-03-08 This invaluable book provides comprehensive coverage of contemporary serological cellular and molecular methodologies in histocompatibility testing and their application to human organ transplantation and transfusion The contributors are internationally respected authorities in histocompatibility and immunogenetics and are closely involved in the development or application of state of the art technologies The first three sections of the book are primarily intended for use as a bench manual for histocompatibility testers immunologists and immunogeneticists the fourth and fifth sections on selection of donors and statistical methods will further assist medical practitioners involved in clinical transplantation and its outcome The final section of the book reviews the genetics and clinical relevance of minor histocompatibility antigens      **The SNU Manual of Surgical Care 5 Edition**

Department of Surgery, Seoul National University College of Medicine, 2024-03-13 This book serves as a concise surgical manual that quickly references the latest information essential for determining the policies of surgical patient care and pre and post operative treatment It is a necessary guide not only for surgical residents and medical students but also for practicing surgeons Features of this book 1 Provides information on surgical care through a systematic approach to surgery related topics 2 Reflects the latest research findings and recommendations on the rapid development and evolution of the surgical field 3 Covers a wide range of topics and areas in surgical surgery including patient management before and after surgery basic techniques and procedures management of complications fluids and nutrition 4 Offers practical guidance that can help in real surgical situations and practice 5 Available in a pocket size that can be easily referred to at any time 6 An English edition that can be referenced by medical professionals both in the Korea and abroad Table of contents Contributors

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