

Chapter 8.2

Cryopreservation of Lymphocytes in Bulk

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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 Me_2SO , 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

Me_2SO : Dimethylsulfoxide
DNase: Deoxyribonuclease

Ashi Laboratory Manual

Elizabeth H. Hammond

A red circular graphic with a gradient, appearing as a stylized arrow or a partial circle, located to the right of the author's name.

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ASHI Laboratory Procedure Manual American Society for Histocompatibility and Immunogenetics, Amy B. Hahn, Geoffrey A. Land, Rosemarie M. Strothman, 2000 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 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 contributions 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 *Kidney Transplantation, Bioengineering, and Regeneration* Giuseppe Orlando, Giuseppe Remuzzi, David F. Williams, 2017-06-08 Kidney Transplantation Bioengineering and Regeneration Kidney Transplantation in the Regenerative Medicine Era investigates how the field of regenerative medicine is changing the

traditional premises of solid organ transplantation specifically within the field of kidney transplantation In Section 1 chapters illustrate the state of the art in kidney transplantation as well as the research behind the bioengineering and regeneration of kidney organoids for therapeutic renal replacement In Section II chapters catalog the technologies that are being developed and the methods that are being implemented to bioengineer or regenerate kidneys in order to restore function while critically highlighting those technological advances which hold the most promise The book thus encompasses clinical renal transplantation tissue engineering biomaterial sciences stem cell biology and developmental biology as they are all applied to the kidney Focuses on the synergy between renal organ transplantation and regenerative medicine highlighting the advances within transplantation bioengineering regeneration and repair Educates the transplant community on important regenerative medicine research pertinent to kidney transplantation Develops a shared language for clinicians surgeons and basic researchers to reach across the fields of transplantation and regenerative medicine and facilitate more productive investigation and research Catalogs the technologies being developed and methods being implemented to bioengineer or regenerate kidneys to restore function *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

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An Introduction to Clinical Immunology and Serology Frances K. Widmann, Carol Ann Itatani, 1998 This text is geared for readers with little or no experience in immunology or clinical medicine and provides an understanding of what the immune system does how it protects the body and how immunologic principles apply to an ever increasing array of laboratory tests

Clinical Transplants, 2009 *Solid Organ Transplantation Pathology* Elizabeth H. Hammond, 1994 This text provides the reader with information and pertinent references on important aspects of solid organ transplant pathology Diagrams are used to clarify concepts and nomenclature Illustrations of light microscopic features of the changing classification of allograft rejection in various organ sites are also provided Topics covered include the actions and

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