

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 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

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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 **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 section has been completely updated to align with the latest International Union of Immunological Societies classifications of inborn errors of immunity Functional Cellular Assays expanded content includes detailed discussions on various functional assays critical for modern immunologic testing Autoimmune Diseases expanded chapters on systemic and organ specific autoimmune disorders including new chapters on Sjögren's syndrome and deficiency of ADA2 as well as significant updates on organ specific autoimmune diseases Transplantation Immunology updated chapters detail the assessment of immune reconstitution and ABO testing reflecting latest practices The 9th Edition of the Manual of Molecular and Clinical Laboratory Immunology serves as an invaluable resource for laboratory directors clinicians laboratory managers technologists and students It provides critical insights into the selection application and interpretation of immunologic tests offering practical guidance on troubleshooting clinical application and an understanding of test limitations This comprehensive and up to date manual remains an essential tool for anyone involved in the diagnosis evaluation and management of immune mediated and immune system related disorders

Immuno-hematology: Principles and Practice Eva D Quinley, 2020-06-15 Immuno-hematology 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

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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

Clinical Laboratory Medicine Kenneth D. McClatchey, 2002 This thoroughly updated Second Edition of Clinical

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