
Preface

Neutrophils [also known as polymorphonuclear leukocytes (PMNs) or granulocytes] are the most abundant white cell in humans. Granulocytes and/or granulocyte precursors normally comprise ~60 % of the nucleated cells in bone marrow and blood. Mature neutrophils have a typical circulating half-life of 6–8 h in the blood and then migrate through tissues for ~2–3 days. Their relatively short life-span is devoted largely to surveillance for invading microorganisms. During infection, the neutrophil life-span is extended, granulopoiesis increases, and large numbers of neutrophils are rapidly recruited to the site(s) of infection. Following recognition (binding) and phagocytosis of microorganisms, neutrophils utilize an extraordinary array of oxygen-dependent and oxygen-independent microbicidal weapons to destroy infectious agents. Oxygen-dependent mechanisms involve the production of reactive oxygen species (ROS), while oxygen-independent mechanisms include degranulation and release of lytic enzymes and bactericidal peptides. Inasmuch as these processes are highly effective at killing most ingested microbes, neutrophils serve as the primary cellular defense against infection.

The aim of *Neutrophils: Methods and Protocols, Second Edition* is to provide (1) a set of protocols to assess most basic neutrophil functions, (2) protocols for investigating specialized areas in neutrophil research, and (3) step-by-step diagnostic assays for common neutrophil disorders. A wide variety of methods have been developed to assess neutrophil function, and these methods have been instrumental in advancing our understanding of the role of neutrophils in host defense and inflammatory disease. For those researchers and clinicians interested in the study of neutrophils, the availability of a comprehensive source of protocols describing the most modern methodological advances in neutrophil biology is invaluable, as many publications do not provide information on the finer details critical to success of a given method. As such, we have compiled a series of protocols written by leading researchers in the field that provide detailed guidelines for establishing and performing the most common neutrophil function assays. Hints of the best way to perform these methods as well as guidance in detecting associated problems are included, so novice investigators will also be able to effectively utilize these assays. While the volume provides current protocols for evaluation of most basic neutrophil functions and certain specialized functions, a section is dedicated to diagnostic assays for common neutrophil disorders. Thus, this volume is designed for the basic researcher involved in the study of neutrophil function and clinical investigators interested in medical aspects of neutrophil function in health and disease.

In the second edition of *Neutrophils: Methods and Protocols* all of the chapters have been updated, including many new approaches. In addition, the *Second Edition* contains a number of new chapters that were not included in the *First Edition*. Part I is an overview of neutrophils and their role in host defense and inflammation. Part II describes the most commonly used methods to isolate neutrophils from humans and other animal species and procedures for subcellular fractionation of human neutrophils. This section also contains a chapter that details collection and analysis of in vivo-transmigrated neutrophils.

Part III encompasses protocols addressing neutrophil biochemistry, electrophysiology, signal transduction, and apoptosis. New chapters covering neutrophil microinjection and generation of mature neutrophils from induced pluripotent stem cells are now included. Part IV details methods for investigating adhesion and chemotaxis, with new chapters on evaluation of neutrophil migration through extracellular matrix and characterization of outside-in signaling via integrins. Part V provides protocols for assessing neutrophil phagocytosis and bactericidal activity, including new chapters that describe how to measure phagocytosis by flow cytometry and analyze formation and function of extracellular traps. Part VI provides an extensive set of assays for evaluating NADPH oxidase priming and activation, production of reactive oxygen species, and new chapters describing analysis of p47^{phox} phosphorylation and flavocytochrome *b* conformational changes during neutrophil activation. Part VII includes protocols to measure gene expression in neutrophils and a new chapter on high-purity neutrophil isolation from saliva for transcriptome analysis. Finally, Part VIII provides assays for diagnosis of the most common neutrophil disorders, including an updated section on assays for myeloperoxidase and myeloperoxidase deficiency. In addition to the step-by-step protocols, the *Notes* section of each chapter provides an outstanding depot of useful and interesting information not typically published in the Methods sections of standard journal articles.

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