

# Fact sheet: Quantitative Determination of Leukocyte Oxidative Burst



## Description

This test quantifies the leukocyte oxidative burst in heparinized whole blood in response to opsonized bacteria (*E. coli*), phorbol 12-myristate 13-acetate (PMA) and the chemotactic peptide N-formyl-Met-Leu-Phe (fMLP). It determines the percentage of phagocytic cells which produce reactive oxidants via conversion of the fluorogenic substrate Dihydrorhodamine (DHR) 123 to R 123 .

## Indication

- Assess the influence of immuno-modulatory therapies on oxidative burst
- In vitro screening of novel compounds which modulate leukocyte functions

## Pathophysiology

Reduced or missing burst activity is observed in inborn defects like the chronic granulomatous disease (CGD). CGD is a heterogeneous group of inherited disorders that usually manifest during the first two years of life (1, 2). Clinically, the disease is characterized by repeated and life-threatening infections by bacterial and fungal organisms. These infections typically comprise pneumonia, lymphadenitis, or abscesses that involve lymph nodes, lungs, and liver.

The NADPH oxidase is the enzyme system responsible for producing superoxide anion, which is quickly converted to hydrogen peroxide and hydroxyl radicals. Abnormalities in the NADPH oxidase enzyme system lead to CGD. Neutrophils from CGD patients fail to produce a significant oxidative burst following stimulation. Different forms of CGD are described (classical X-linked CGD and autosomal recessive patterns).

The oxidative burst of granulocytes is impaired in transplant patients and patients with AIDS (3). The spontaneous and fMLP-induced neutrophil respiratory burst is increased in neonates with laboratory signs of infection (4).

Various immunomodulators (e.g., cytokines (GM-CSF, G-CSF, TNF) or drugs) affect the oxidative burst. By using fMLP as a low stimulant one can investigate additive or priming effects (5) of test substances.

## Method

- Flow cytometric assay

## Sample

- Heparinized whole blood (do **not** use EDTA or Citrate)

## Preanalytics

- Standard

## References

- (1) Donadebian HD. Congenital and acquired neutrophil abnormalities. In: Klempner, M.S. et al. (eds) Phagocytes and Disease. Kluwer, Dordrecht Boston New York, 1989, pp 103-118
- (2) Smith, RM and Curnutte JT Molecular basis of chronic granulomatous disease. *Blood* 1991, 77: 673 -686.
- (3) Dohmeyer TS et al. Decreased function of monocytes and granulocytes during HIV-1 infection correlates with CD4 cell counts. *Eur J Med Res* 1995, 1: 9-15.
- (4) Gessler P et al. Neutrophil respiratory burst in term and preterm neonates without signs of infection and in those with increased levels of C-Reactive Protein. *Pediatr Res* 1996, 39: 843-848.
- (5) Elbim C et al. Priming of polymorphonuclear neutrophils by tumor necrosis factor in whole blood: Identification of two polymorphonuclear neutrophil subpopulations in response to formyl-peptides. *Blood* 1993, 82: 663-640.