RAPID FLOW CYTOMETRIC TEST USING EOSIN-5-MALEIMIDE FOR DIAGNOSIS OF RED BLOOD CELL MEMBRANE DISORDERS

Kalaya Tachavanich, Voravarn S Tanphaichitr, Wiyakan Utto and Vip Viprakasit

Hematology/Oncology Division, Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Abstract. Conventional diagnosis of hereditary red blood cell (RBC) membrane disorders, in particular hereditary spherocytosis (HS), is labor intensive, time consuming and requires at least 2 ml of blood, which might be impractical in neonatal period. We evaluated the use of eosin-5-maleimide (EMA), a dye that reacts covalently with lysine-430 on the first extracellular loop of band 3 protein, for rapid screening test of patients with HS and Southeast Asian Ovalocytosis (SAO). Fresh RBCs from 142 healthy controls, 50 HS, 17 SAO, 29 hereditary elliptocytosis, 5 autoimmune hemolytic anemia, 66 patients with β-thalassemia/HbE, 31 cases with α-thalassemia (HbH disease) and 4 cases with pyruvate kinase deficiency were stained with EMA, and analyzed for their mean channel fluorescence (MCF) using a flow cytometer. RBCs from patients with HS and SAO expressed a greater degree of reduction in MCF compared to those from normal controls and other hemolytic diseases. These findings showed that the fluorescence flow cytometric-based method is a simple, sensitive and reliable diagnostic test for RBC membrane disorders using a small volume of blood, and results could be obtained within 2 hours. Such method could serve as a first line screening for the diagnosis of HS and SAO in routine hematology before further specific membrane protein electrophoresis and molecular diagnosis are employed.

Correspondence: Kalaya Tachavanich, Hematology/Oncology Division, Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Pran Nok Road, Bangkok Noi, Bangkok 10700, Thailand.
Tel: 66 (0) 2419 5973; Fax: 66 (0) 2866 3021
E-mail: kalayata@yahoo.com