

Thesis Title Two Red Cell Population Detection
by Fluorescent Microsphere Method

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ABSTRACT

Two populations of red blood cells (RBCs) are conditions found in erythrocyte chimerism in patients after bone marrow transplantation (BMT), survival of donor erythrocytes and antigen positive fetal erythrocytes in maternal blood. The blood group antigen difference between patient and donor was used to demonstrate the mixed chimerism. The fluorescent microsphere method was applied for the detection of two RBC populations in patient by using fluorescent microspheres coated with anti-human IgG or monoclonal IgG antibody. Blood sample from patient after allogeneic BMT was sensitized with human IgG antibody specific for

antigen presented on donor RBCs. After washing, the RBCs were incubated with anti-human IgG coated fluorescent microspheres. When the source of IgG antibody was not human antibody, the donor RBCs were directly stained with IgG antibody coated fluorescent microspheres. The selected blood groups were based on the distribution and strength of antigens in addition to the availability of the potent antisera. The A₁, B and E were employed in the study cases and D antigen in the study of optimal conditions. The comparison between fluorescent microsphere method and differential agglutination technique showed that the fluorescent microsphere method was able to detect the antigen positive red cells at the level of 0.3 percent which it yielded more sensitive result than differential agglutination technique. Mean linear regression line for mixtures of the antigen positive and negative RBCs in various proportions ranging from 5.0 to 80 percent was $y = 0.801x + 0.949$. At the concentration of antigen positive RBCs lower than 2.5 percent, linear regression equation was $y = 0.448x + 0.61$. The correlation coefficient (r) between the fluorescent microsphere method and differential agglutination for mixtures of the antigen positive and negative red cells ranging from 5.0 to 80 percent and lower than 2.5 percent were $r = 0.99$ ($p < 0.0005$) and 0.98 ($p < 0.05$), respectively. The correlation coefficient (r) between the measurement

of mixtures of the antigen positive and negative RBCs in various proportion ranging from 0.6 to 8 percent measured by fluorescence microscope and flow cytometer was 0.98 ($p < 0.0005$). The linear regression equation was $y = 0.985x + 0.065$. In three patients after allogeneic BMT, one of them showed mixed chimerism in the ratio of recipient : donor RBC 5:3 at 8 months. This study showed that the fluorescent microsphere can be to establish the engraftment of donor marrow and investigate mixed chimerism after transplantation. However the use of highly selected potent antisera is recommended in order to generate the good and reliable result.