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***Short Communication***

# A RAPID METHOD FOR STARTING A CULTURE FOR THE ESTABLISHMENT OF EPSTEIN-BARR VIRUS-TRANSFORMED HUMAN LYMPHOBLASTOID CELL LINES

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*Summary* We developed a simple and efficient procedure for the establishment of Epstein-Barr virus-transformed human lymphoblastoid cell lines. B-lymphocytes were obtained by centrifugation after hemolysis of red cells with a hemolysis buffer, instead of Ficoll-Parque gradient. We can start a primary culture within 15 min by using this method.

***Key Words*** cell culture, EB virus-transformed lymphoblastoid cell lines, a rapid method

Permanent B-lymphoblastoid cell lines (BLCL) obtained by transforming peripheral blood B-lymphocytes using Epstein-Barr virus (EBV) are of great practical value in biochemical, cytogenetic and molecular researches (Neitzel, 1986). Especially, BLCL are ideal sources for molecular study in human as DNA samples can be easily and repeatedly prepared from them. One of the difficulties had been a low success rate in establishing BLCL, but the use of cyclosporin A, an immunosuppressive agent, increased success rates up to 95% (Anderson and Gusella, 1984). Another problem for obtaining BLCL as a routine use includes that the procedure is time-consuming. It takes nearly 2 hr to initiate a primary culture.

We report a simple and efficient procedure for a routine and practical use. We can start a primary culture within 15 min by using this hemolysis method.

One to 5 ml of heparinized blood samples was collected and held at ambient temperature for 0 to 72 hr during shipment to the laboratory. One volume (usually 5 ml) of the blood sample was treated with three volumes (usually 15 ml) of hemolysis buffer (155 mM $NH_4Cl$, 10 mM $KHCO_3$, 0.1 mM EDTA, pH 7.4) and 5 $\mu$l/ml

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(usually 100 $\mu$l) of 10% SDS and shaken gradually for 30 to 60 sec. After centrifugation at 1,000 rpm for 5 min, a cell pellet was resuspended in RPMI1640 medium and washed once by pelleting (1,000 rpm, 5 min). The final cell pellet was resuspended in the primary culture medium (RPMI1640 with 20% fetal bovine serum, 2 mM L-glutamine, antibiotics, and 10 $\mu$g/ml cyclosporin A). Finally, 1/10 volume of an EBV stock solution, which had been prepared by filter sterilizing supernatant fluid from 1-week-old saturated cultures of B95-8 marmoset cells, was added. The volumeof the primary culture medium and the EBV stock solution was the same as that of the blood samples. Culture flasks (usually, Costar 3050, or Nunclon tube in the case of less than 2 ml) received the cells resuspended in the medium were set vertically in a 37°C, 5% $CO_2$ incubator. After 7 days, a half of the supernatant was removed and replaced by fresh culture medium (RPMI1640 with 10% fetal bovine serum, 2 mM L-glutamine and antibiotics). No additional cyclosporin A was added. Thereafter, a half of the medium was changed twice a week. When large clumps appeared (usually 2 to 3 weeks after starting the culture), cultures were fed by doubling the volume of the medium every 3 to 4 days. Then subculturing, freezing and harvesting the cells were carried out according to the need.

Following this protocol, BLCL were successfully established in 200 (98.0%) of 204 samples in our laboratory during May, 1991 and January, 1992. The samples were obtained from patients and their families of malformation syndromes, chromosome aberrations and single gene disorders. These BLCL were used for molecular research of these disorders.

The hemolysis buffer of this protocol is originally used for hemolysis before DNA extraction from peripheral blood (Baas *et al.*, 1984). Treatment with this hemolysis buffer was not harmful for B lymphocytes of peripheral blood. The successful establishment of BLCL was achieved even if the volume of samples was as small as 1 ml, and a delay of starting culture was as late as 4 days after blood sampling.

This method reduces time to spend for cell culture and is convenient for a routine use.

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