Freezing and Rejuvenation of Human O Erythrocytes for Use in the Immune Adherence Hemagglutination Test

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A method was established to freeze selected human O erythrocytes and to thaw them as necessary for use in the immune adherence hemagglutination test. This method ensured interrun reproducibility and eliminated the necessity to screen blood donors (fresh cells) for acceptable C3b receptor site sensitivity.

The Serology Section of the Virology Division, Michigan Department of Public Health, performs an immune adherence hemagglutination test for detection of various antiviral antibodies. The daily workload requires 20 to 40 microtiter plates per day and 1 to 2 ml of packed human O erythrocytes from a suitable donor. Substantial labor and expense are required to ensure interrun reproducibility among batches of fresh cells from different donors. Variation occurs in human O cell C3b receptor sites due to both differing sensitivity among blood donors and the effect of prolonged storage of the cells at 4°C (2, 4). Therefore, a method was established for freezing erythrocytes in glycerol, storing them at −70°C, and rejuvenating them as needed (6). With this method, a unit of blood can be frozen in aliquots of a convenient volume and the same blood used on a weekly basis for 6 months. The method has improved the reproducibility of results obtained with the 10 to 20 viral antigens used routinely in the immune adherence hemagglutination test.

Erythrocytes from a suitable donor were collected in Alsever solution, and convenient volumes were centrifuged at 500 × g for 5 min at room temperature. The supernatant fluid was removed, and the cells were resuspended in fresh sterile Alsever solution at a 20% cell concentration and again centrifuged as described above. The supernatant fluid was removed, and this time the cells were suspended to a 20% cell concentration in minimal essential medium with Earle salts (EMEM) (M.A. Bioproducts, Walkersville, Md.), pH 7.2 to 7.4, containing 30% glycerol. The suspended cells were equilibrated to room temperature for 10 min, to 4°C for 20 min, and then frozen in centrifuge tubes (Corning Glass Works, Corning, N.Y.) so that each tube contained a 1-week supply of cells. No special freezing process was used. Tubes were placed in a rack at −70°C and stored until needed.

Cells were removed from the −70°C freezer and thawed in a 37°C water bath for 15 min. Subsequent steps were carried out at room temperature. Two sterile stock solutions were used to remove the glycerol from the cells: 4× EMEM and 1× EMEM, both at pH 7.2 to 7.4. Glycerol was removed by a modification of the method of Meryman and Hornblower (6), using an IEC PR-6000 centrifuge for stepwise removal. EMEM was used as a wash solution for convenience. The thawed cell suspension was divided into two centrifuge tubes. An equivalent amount of 4× EMEM was added to each tube to give a 1:2 dilution. Both tubes were mixed and centrifuged at 1,000 × g for 5 min at room temperature. The supernatant fluid was removed, and the cells were resuspended in 4× EMEM at a 40% cell concentration. After 1 to 2 min for equilibration, 1× EMEM was added to make a 20% cell concentration. After another 1 to 2 min for equilibration, the cell suspension was centrifuged at 500 × g for 5 min at room temperature. The supernatant fluid was removed, and the cells were suspended in Alsever solution at a 20% cell concentration for use. Cells could be stored at 4°C for as long as 1 week.

The immune adherence hemagglutination test was performed as previously described (5). Frozen cells were stored at −70°C for 3 months. Cells from the same donor were compared in two ways. Frozen rejuvenated cells were compared with fresh cells immediately after thawing and after both fresh and rejuvenated cells had been stored for 1 week at 4°C. Twelve different viral antigens were used in the comparisons. These included influenza A and B NP antigens, adenovirus, cytomegalovirus, parainfluenza 3, respiratory syncytial, herpes simplex, varicella-zoster, eastern equine encephalitis, St. Louis encephalitis, measles, and mumps viruses. Known reactive and nonreactive control sera for each antigen and paired sera with four-fold rises in titer were used to determine serum titers with both types of cells (3). Titers were the same in all cases, regardless of the cells used or the length of storage. One week of storage in Alsever solution at 4°C did not affect the performance of either fresh or rejuvenated cells.

These results indicate that the C3b receptors remain relatively intact on the erythrocytes during the freezing and rejuvenating processes, even though some 10 to 15% erythrocyte lysis occurred.

Frozen rejuvenated cells may also be beneficial in other tests in which sensitivity of the test depends upon the C3b receptors used, as in the enhanced hemagglutination inhibition test (1). It appears that the use of frozen rejuvenated cells has helped substantially to eliminate a test variable from the immune adherence hemagglutination technique. Interrun reproducibility has improved, and the work necessary for frequent screening of erythrocyte donors has been eliminated.

LITERATURE CITED
tion, Inc., Washington, D.C.