Application of Freeze-Dried Monkey Erythrocytes to Measles Viral Hemagglutination and Hemagglutination-Inhibition Tests

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Erythrocytes collected from monkey species including grivet, rhesus, and cynomolgus monkeys were stabilized by fixation with glutaraldehyde of a low concentration and were freeze-dried in vacuo. These freeze-dried erythrocytes were compared with fresh erythrocytes for measles viral hemagglutination and hemagglutination inhibition and could be used in both tests instead of fresh erythrocytes. They maintained their initial appearance and sensitivity to measles viral hemagglutinins after storage at 4°C for 4 months more.

The hemagglutination-inhibition (HI) test for measles antibody has proved to be a valuable tool in the serological diagnosis of measles. The HI test is more reproducible and easier to perform than neutralization and complement fixation (CF) tests. Measles virus can agglutinate only primate erythrocytes (RBC), which include those of baboons (*Papio unibas*) and various species of monkey. The most commonly used are those of rhesus monkeys (*Macaca mulatta*), and probably the best are those of grivet monkeys (*Cercopithecus aethiops*) because of sensitive and reproducible results (3). However, it is very difficult to obtain such monkey RBC in an emergency or for incidental use. Thus, we attempted to stabilize the RBC of several species of monkey so that they could be stored for long periods and used in routine assays.

Monkeys were bled from the femoral vein by using an equal volume of sterile Alsever solution as an anticoagulant; the blood was stored at 4°C. Before stabilization, the RBC were washed three times with 0.067 M phosphate-buffered saline (pH 7.2) and suspended in the same solution at a concentration of 1% (vol/vol). Ten volumes of the RBC suspension were then mixed with 1 volume of 0.1% glutaraldehyde (GA) diluted with phosphate-buffered saline. For fixation, the mixture was gently agitated at room temperature for 60 min. The fixed cells were washed six times with 10 volumes of phosphate-buffered saline to remove unabsorbed GA and then suspended in a 5% lactose solution to a concentration of 10%. This suspension was rapidly frozen in a mixture of solid carbon diox-

ide and acetone and then dried in vacuo. For comparison, formalinized RBC were prepared by similar procedures.

Measles viral hemagglutinins (HA) were prepared from the Toyoshima strain of measles virus in Vero cells (2). The HA were treated with Tween 80 and ether before use.

Serum specimens were obtained from institutionalized children before and at various intervals after clinically typical measles infections. Specific hyperimmune sera were produced in guinea pigs by injecting, intramuscularly, a mixture of virus material and complete Freund adjuvant. These sera were stored at -20°C until tested.

The serum specimens were tested for both HI and CF antibodies. The CF procedure was that described by Bussell et al. (1), employing two full units of complement, eight units of antigen, and overnight fixation at 4°C. HA and HI tests were carried out by the microtiter method of Sever (8), using V plates of the permanent type (Tomy Seiko Co., Tokyo, Japan) and spiral loops (Microbiological Associates Inc., Bethesda, Md.). Phosphate-buffered saline (0.067 M, pH 7.2) containing 0.1% bovine serum albumin and 0.001% gelatin was used throughout as a diluent for serum, HA, and RBC suspensions. Freeze-dried RBC were used as 0.4% suspensions, and fresh RBC were used as 0.3% suspensions. Nonspecific inhibitors were removed from the serum by treatment with 25% acid-washed kaolin (Fisher Scientific Co., Pittsburgh, Pa.) by the method of Rosen (6).

The GA-fixed cells were bright red immediately after fixation and became slightly brown-

ish with succeeding lyophilization. The final color, however, was lighter than that of formalinized erythrocytes.

Although grivet monkey RBC fixed with 0.37% formaldehyde were agglutinated by measles HA at titers as high as 128, they easily hemolyzed when suspended in water (Table 1). The RBC fixed with more than 0.37% formaldehyde became less sensitive to measles HA. This result is compatible with that of Peries and Chany (4). However, GA was effective for stabilizing the monkey RBC without losing their agglutinability, probably because GA was a bi-functional aldehyde and could unite one membrane constituent with the other on the same cell surface, thus resulting in stabilization of the RBC at such a low concentration that the receptor substances were not destroyed.

There was no significant difference in HA titers between fresh and freeze-dried RBC, and freeze-dried RBC maintained their original sensitivity to measles viral HA even after storage at 4°C for 4 months (Table 2). When paired sera from a hyperimmunized guinea pig and six children infected with measles were tested for measles HI antibody, there was no difference in HI titers between fresh and freeze-dried RBC

TABLE 1. Effect of various concentrations of GA or formaldehyde (FA) on measles viral HA titer and the stability of monkey RBC

Source of RBC	Aldehyde	Concn of aldehyde added (%)	HA titer ^a	Hemolysis ^b
Grivet			128	++
	GA	0.05	128	+
	GA	0.10	128	—
	GA	0.25	32	—
	GA	0.50	4	—
	FA	0.37	128	++
	FA	3.7	64	+
Rhesus	FA	18.5	4	—
			64	++
	GA	0.05	128	+
	GA	0.10	64	—
	GA	0.25	16	—
Cynomolgus	GA	0.75	4	—
			32	++
	GA	0.05	32	+
	GA	0.10	32	—
	GA	0.25	8	—
	GA	0.75	4	—

^a Reciprocal of antigen titer.

^b Hemolysis of fresh and aldehyde-fixed cells suspended in water. Symbols: ++, strongly positive reaction; +, positive reaction; —, negative reaction.

TABLE 2. Stability of freeze-dried monkey RBC compared with that of fresh RBC by measles viral HA tests

Source of RBC	HA titer ^a			
	Fresh RBC	Freeze-dried RBC stored for:		
		2 weeks	1 month	4 months
Grivet	128	128	64	128
Rhesus	64	64	64	32
Cynomolgus	32	16	16	32

^a Reciprocal of antigen titer.

from grivet monkeys; moreover, the increase in the HI antibody titers in the convalescent sera was of the same magnitude as with the CF tests.

We used the RBC of rhesus and cynomolgus (*Macaca irus*) monkeys in addition to those of grivet monkeys. Both of them were also successfully stabilized by GA (Table 1).

The contrast between agglutinated and non-agglutinated RBC patterns, obtained with freeze-dried RBC, was not so sharp as that produced in the HA test with fresh RBC. However, when freeze-dried RBC were used at a higher concentration (0.4%) than fresh RBC (0.3%), the difference became comparable to that obtained with fresh RBC. In addition, sharp end points similar to those of freeze-dried RBC were noted when the tests were allowed to stand overnight at 4°C after incubation at 37°C for 2 h. When the HI tests were carried out by the test tube method of Rosen (7), which was routinely adopted, this advantage was apparent.

Thus, successful stabilization of monkey RBC without loss of sensitivity to measles HA made RBC more readily available for routine tests. Further studies will be conducted to test the stability and dependability of the freeze-dried monkey RBC over a prolonged period of time.

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