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CRYOPRESERVATION OF THE BLOOD STAGES OF *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX* FOR IN VIVO STUDIES*

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Abstract. Cryopreservation in Alsevers-glycerol of the blood stages of *Plasmodium falciparum* (5 strains) and *P. vivax* (2 strains) indicated that parasite infectivity for *Aotus* was retained for more than 1,100 days.

The need for a reliable and reproducible method of cryopreservation of malaria parasites, especially *Plasmodium falciparum*, has been noted.[1] Various procedures have been reported for the cryopreservation of *P. falciparum*-infected blood, either with no preservative,[2,3] the addition of glycerol,[4–7] or dimethylsulfoxide.[8] The only report using Alsevers solution with glycerol (10%) and low temperature preservation referred to *P. berghei*.[9]

The present report summarizes our experience using Alsevers solution and 7.5% glycerol for the cryopreservation of various strains of *P. falciparum* and *P. vivax*.

MATERIALS AND METHODS

Both donor monkeys, from which parasites were obtained for cryopreservation, and the recipients were *Aotus trivirgatus* captured on the Isthmus of Panama.

The preservative medium consisted of 9 parts Alsevers solution to 1 part glycerol; 1.5 ml of this mixture was transfered to 5-ml Vacutainer® tube, autoclaved and stored at 5°C. Citrated blood (0.5 ml) from a donor monkey was added to each tube, and the mixture was immediately shell frozen in a dry ice and alcohol slurry. The tubes were stored in a mechanical freezer at −70°C. The cryopreserved material was thawed rapidly in cold running tap water and inoculated intraperitoneally into a monkey.

RESULTS

Five cryopreserved strains of *P. falciparum*, and 2 strains of *P. vivax* (Table 1) were inoculated into *Aotus*. Parasites of the Vietnam Oak Knoll strain of *P. falciparum* remained viable after 1,105 days of cryopreservation, while Chesson *P. vivax* parasites retained viability for as long as 1,123 days of cryopreservation.

Prepatent periods varied from 2 to 28 days in the inoculated monkeys; there was no relationship between the parasitemia at the time of cryopreservation and the length of the prepatent period.

A sample of *P. falciparum* (Malayan Camp strain) that containing 30,000 parasites/cmm and cryopreserved for 346 days had a sufficient number of viable parasites to infect an *Aotus*. As few as 10,000 parasites/cmm, when cryopreserved, of *P. vivax* (Achiote strain) remained infective after 181 days.

Two failures to recover viable *P. falciparum* parasites occurred: a sample of the Vietnam Smith strain containing 206,000 parasites/cmm that had been cryopreserved for 322 days, and a sample of the Panama II strain, that contained 101,000 parasites/cmm and had been cryopreserved for 149 days.

DISCUSSION

Our trials were successful with *P. falciparum* (Vietnam Oak Knoll strain) parasites cryopreserved for 1,105 days and *P. vivax* (Chesson

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TABLE 1

Positive revival of Plasmodium falciparum *and* P. vivax *strains after cryopreservation in Alsevers-glycerol*

Malaria strain	No. of *Aotus* inoculated	No. of days cryopreserved mean (range)	Parasitemia per cmm × 10³* mean (range)	Prepatent period-days mean (range)
		Plasmodium falciparum		
Uganda Palo Alto	10	339 (83–725)	1,061 (498–1,971)	7 (2–15)
Vietnam Oak Knoll	6	467 (96–1,105)	1,377 (63–1,952)	6.2 (6–7)
Panama II	4	121 (61–299)	996 (773–1,157)	17 (8–28)
Malayan Camp	3	337 (6–660)	1,268 (30–236)	10.7 (2–17)
Vietnam Smith	2	492 (433–551)	443 (175–711)	10 (5–15)
		Plasmodium vivax		
Achiote	3	104 (7–181)	30 (10–70)	15 (8–22)
Chesson New Guinea	2	691 (222–1,123)	83 (82–84)	5.7 (2–8)

* At the time of cryopreservation; each sample contained 0.5 ml of infected blood.

strain) cryopreserved for 1,123 days. The Camp strain of *P. falciparum* was reported to retain its viability in a chimpanzee after storage in liquid nitrogen for 968 days,[5] and the McClendon strain of *P. falciparum*, after 1,921 days of storage at −78°C, was infective for man.[3] The Chesson strain of *P. vivax*, cryopreserved in liquid nitrogen for 2,860 days, was infective for an *Aotus*.[10]

The highly reliable technique of using Alsevers-glycerol (7.5%) to cryopreserve *P. falciparum* and *P. vivax* parasites for in vivo utilization was shown by the fact that failures to induce a patent parasitemia occurred in only 2 of 34 attempts. Since thawed samples were inoculated intraperitoneally, deglycerolization was not required as it would have been if the intravenous route of inoculation were used. No apparent adverse reaction in *Aotus* was observed from the glycerine.

Citrated blood from a donor monkey, infected with *P. falciparum* or *P. vivax*, adapted to Panamanian *Aotus*, has initiated patent infections in all inoculation trials (more than 800).

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