

In vitro Infection Rate of *Plasmodium falciparum* in Human
Erythrocytes with Different Hemoglobin Types

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OBJECTIVE: To compare parasite densities in human erythrocytes of different hemoglobin types when cultured *in vitro*.

BACKGROUND: It has been proposed that resistance to *P. falciparum* infection is related to different natural factors. Among these, the red blood cell defects, namely Hb S, Hb E, thalassemia and G-6-PD deficiency have been extensively studied.¹⁻⁷ Evidence supporting the theory that Hb S deficiency conferred natural resistance to the host were convincing, but controversial results were obtained in thalassemia and Hb E⁸.

Significant numbers of individuals with different hemoglobin types and thalassemia exist in Thailand. A number of such cases are available at the Division of Hematology, Department of Medicine, Siriraj Hospital. Extensive studies on hematological aspects of hemoglobin differences have been performed at the Division. A collaborative study has been designed to study these differences utilizing the capability of *in vitro* culture techniques developed at this laboratory.

Heparinized blood from patients with native infection of *P. falciparum* is the source for parasites. Blood group and hemoglobin type are established and hematological investigations are performed on each case. Compatible uninfected blood with the various hemoglobin types are included in *in vitro* culture serving as target for reinvasions. The proportion of erythrocytes containing different hemoglobin types in each culture will be determined.

The proportion of cells in each *in vitro* culture is prepared as follows:

	Row 1	Row 2
Patient infected cells (AA)	1 volume	1 volume
Target cells (AA)	1 volume	—
Recipient cells (AE)	—	1 volume
% of AA cells	100	50
% of AE cells	—	50

Erythrocytes containing different Hb types can be differentiated, e.g., Hb E containing erythrocytes and Hb F containing erythrocytes can be distinguished by the technique of Hb staining by differential elutions. The parasite density rate of different hemoglobin containing erythrocytes can be determined by counter-staining with Giemsa Stain.

If the results obtained from such cultures reveal a lower *in vitro* infection rate of thalassemic erythrocytes or erythrocytes containing abnormal hemoglobin, it will suggest that intraerythrocytic conditions of these cells will not readily support the parasite. However, if an equal infection rate is found, a conclusion

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against this hypothesis of protection cannot be made. The selective mechanism against malaria infection may be operating outside the red blood cells or by a combination of the red blood cells and other organs, e.g., the reticuloendothelial system, especially the spleen.

To avoid such controversial results, our experiments are designed differently from the previous described *in vitro* systems.⁹ A later stage of *P. falciparum* trophozoites will be used in our study to allow a longer period of observation of the young merozoites after reinvasion.

PROGRESS: Collections of infected blood are being made, A portion of each sample is cultured immediately while the remainder is frozen in liquid nitrogen for later comparison. The initial experiments are to determine if freezing will have an effect on cells with different types of hemoglobin. The infected cells of certain hemoglobin types are difficult to obtain at some times of the year, and therefore adequate preculture storage methods are necessary.

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