

Investigations of the Fluorescent Spot Test for Erythrocyte Glucose—6—Phosphate Dehydrogenase
Deficiency in Southeast Thailand

Principal Investigator : Ben F. Castaneda, SFC

Associate Investigators : Edward J. Colwell, LTC, MC
Pung Phintuyothin, MG, MC (ret.)
Robert L. Hickman, MAJ, VC

OBJECTIVE: Several investigations of the prevalence of erythrocytic glucose—6—phosphate dehydrogenase (G6PD) deficiency have been reported among male residents of the northern, central and southeastern areas of Thailand. No studies of this genetically determined, sex-linked enzyme deficiency have been accomplished in the remote southeastern regions bordering Cambodia. Both falciparum and vivax malaria are highly endemic in this region and infected patients are often administered primaquine therapy. Since primaquine among other drugs is known to precipitate acute intravascular hemolysis in subjects whose red blood cells are deficient in G6PD activity, it was considered desirable to obtain information on the prevalence of this enzyme deficiency in Southeast Thailand. This report also describes studies on the reproducibility and reliability of the screening technique employed.

DESCRIPTION: The surveys were conducted in Trad Province at the 2 localities shown in Fig. 1. These locations were chosen because of the relative stability of their population and the endemicity of vivax and falciparum malaria in both communities. The capital city of Trad Province has a population of approximately 10,000 people. Takum, a typical rural village with an approximate population of 400 people, is located 18 km northeast of Trad city.

Blood specimens were obtained from the majority of the total male population (i.e., 200) at Takum during a civic action medical patrol. Trad city, which has an estimated male population of 5,000 was arbitrarily divided into 4 geographic regions. Blood specimens were obtained by house visitation from approximately 10% of the estimated male population in each region. All subjects were between the ages of 6 to 60 years and did not exhibit symptoms of clinical illness at the time of specimen collection. Specimens consisted of a heparinized capillary tube of blood obtained by digital puncture. Those obtained at Takum were transported immediately on wet ice to a base laboratory located at the provincial hospital in Trad city. Processing of all specimens was accomplished within 2—3 hours after digital puncture.

The method employed for screening was a commercially available kit of the fluorescent spot technique (Hyland G6PD screen test). The test was conducted in strict accordance with the directions supplied with the kit. Preliminary studies were initially performed to determine the reproducibility of the test. Replicate specimens from 10 male subjects were coded and processed by 3 different technicians. The reactions of the test were observed independently by 3 individuals using the same ultraviolet light source and the results were recorded as strong (bright fluorescence).

Investigations were also undertaken to assess the reliability of the visual observations of the fluorescent spot test. Thirty-eight specimens processed with the spot test were examined for quantitative erythrocytic G6PD activity (G6PD Stain Pack). In addition, the specimens were examined for the proportion of G6PD normal and deficient erythrocytes by the methemoglobin elution test as modified by Gall, et al. (1965). In the latter technique, a mixture of normal and deficient erythrocytes can be differentially identified by a series of chemical treatments which include methemoglobin reduction and elution, and subsequent hematoxylin staining to identify the normal and deficient red blood cells.

PROGRESS: The results of the fluorescent spot test reactions among residents of Trad city and Takum are shown in Table 1. The instructions of the Hyland test kit specify that specimens having no or barely detectable fluorescence are indicative of erythrocytic G6PD deficiency. According to these criteria, the deficiency rates at Trad city and Takum combining weak and negative reactions, were 16.0 and 12.8%, respectively.

The results of replicate examinations for assessing the reproducibility of the fluorescent test are presented in Table 2. In general, there was satisfactory agreement among the results of the replicate examinations and among independent observations of the same reaction by different technicians.

In order to assess the reliability of the spot test results, 2 other methods for analysis of G6PD activity were conducted. Table 3 shows the results of the methemoglobin elution test and the quantitative assay for assessing the reliability of the fluorescent spot test reactions in paired specimens. All specimens exhibiting either a strong or negative reaction in the spot test demonstrated corresponding normal or deficient reactions in the elution test and the quantitative assay. However, of 14 specimens exhibiting weak fluorescent reactions in the spot test, 10 were normal and 4 were deficient in the elution test as well as the quantitative assay.

The results obtained in these alternative tests confirm the reliability of the negative and strong spot test reactions. However, they indicate that the weak reactions are not necessarily indicative of G6PD deficiency. Therefore it is not possible to classify these reactions, and the results of our survey must be presented as a range: 2% to 12% at Takum and 8% to 16% at Trad city. The lower limits of the range denote the proportion of negative reactions in the spot test and the upper limits are the combined proportions of the negative and weak reactions. It is proposed that the G6PD spot test be re-evaluated in the laboratory before further use in the field is considered.

SUMMARY: A survey to determine the prevalence of G6PD deficiency was conducted in Trad city and the village of Takum situated in Trad Province, Thailand employing the fluorescent spot test as the screening technique. The results are reported as a range of 2 to 12% deficiency at Takum and 8 to 16% deficiency at Trad city. Expression as a range of G6PD deficiency was necessitated because of the unreliable interpretations of weak fluorescent reactions in the spot test. A very high degree of reliability of strong and negative fluorescent test reactions was established by the confirmatory results of the quantitative assay and methemoglobin elution tests.

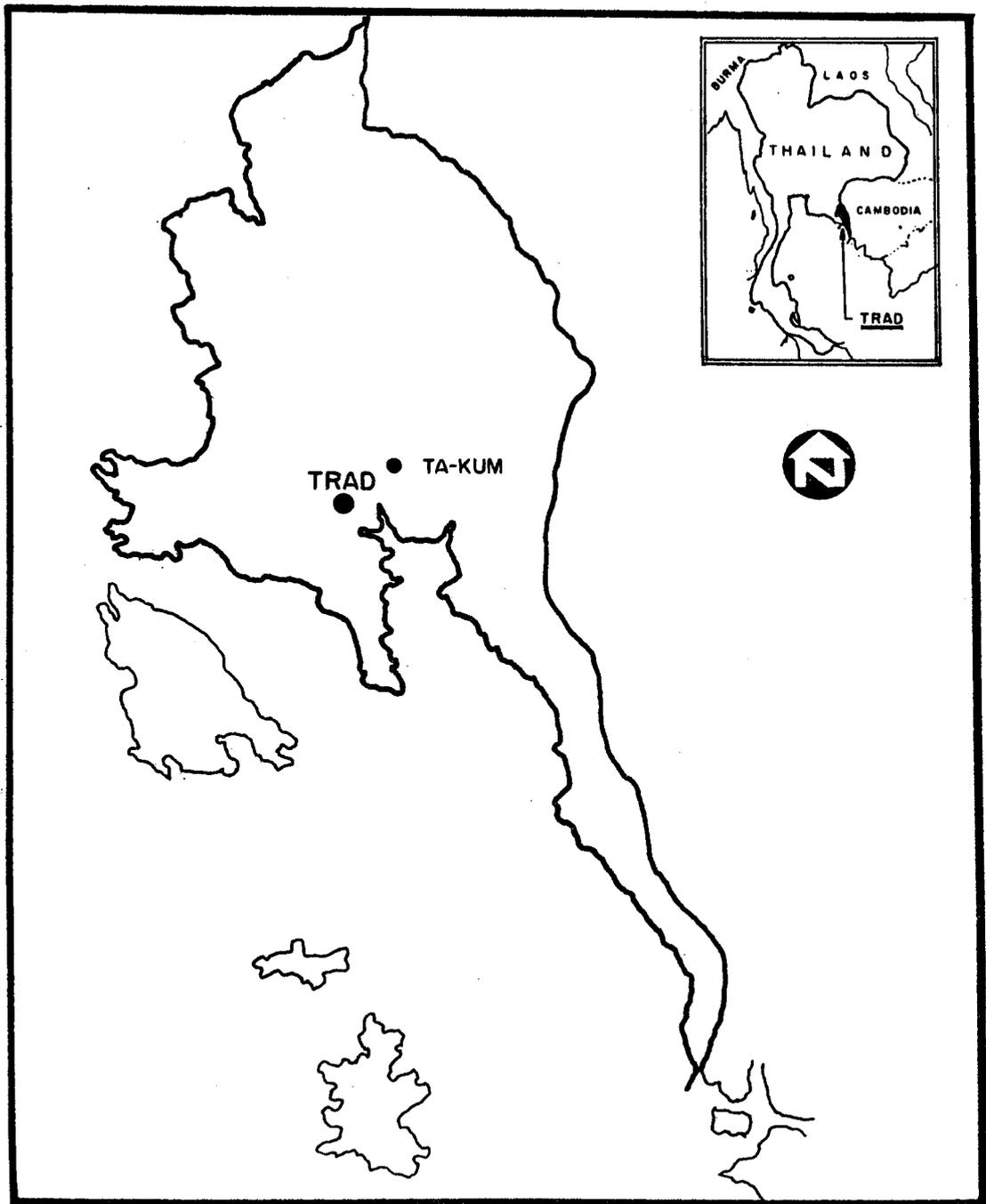


FIG. 1 MAP OF TRAD PROVINCE IN SOUTHEAST THAILAND SHOWING THE STUDY SITES.

Table 1.
Results of the fluorescent spot test reactions in Trad Province.

Location	No. males examined	Rates (%) of spot test reactions		
		Negative	Weak	Strong
Trad City	518	8.3	7.7	84.1
Takum	125	2.4	10.4	87.2
Total	643	7.2	8.1	84.8

Table 2.
Results of eeplicate examinations to access the reproducibility of the fluorescent spot test.

Patient	Replicate A			Replicate B			Replicate C		
	R1*	R2	R3	R1	R2	R3	R1	R2	R3
1	S**	S	S	S	S	S	S	S	S
2	W	W	W	W	W	W	W	W	W
3	S	S	S	S	S	S	S	S	S
4	S	S	S	S	S	S	S	S	S
5	S	S	S	S	S	S	S	S	S
6	W	W	W	S	S	S	W	W	—
7	S	S	S	S	S	S	S	S	S
8	S	S	S	S	S	S	S	S	S
9	—	—	—	—	—	—	—	—	—
10	S	S	S	S	S	S	S	S	S

* Reading of spot test by different technicians

** (S,W,—) indicate strong, weak or no fluorescence, respectively.

Table 3.
Comparison of the results of the fluorescent spot test, methemoglobin elution test and quantitative assay tests.

<u>Spot test</u>		<u>Elution test</u>		<u>Assay test</u>	
<u>Reaction</u>	<u>No. reacting</u>	<u>Normal</u>	<u>Deficient</u>	<u>Normal</u>	<u>Deficient</u>
Strong	21	21	0	21	0
Weak	14	10	4	10	4
Negative	<u>3</u>	0	3	0	3
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