



## CHAPTER I

### INTRODUCTION

#### BACKGROUND AND RATIONALE

Both  $\beta$  thalassemia major and double heterozygous  $\beta$  thalassemia/hemoglobin E can give rise to a serious social problem because the cost for their treatment is considerably high. Therefore, some efforts have been made to prevent these two diseases such as pre-marital counselling, mass screening and pre-natal diagnosis.

The most commonly laboratory tests used for screening purposes are Mean Corpuscular Volume (MCV) and Osmotic Fragility Test (O.F.T.). Unfortunately, these two tests are not practicable for use in the field and therefore, this study is trying to modify O.F.T. so that it can be used to overcome that problem.

Three modifications have been made in this study namely: *First*, the volume of blood sample and reagent (0.36 % buffered saline) - the previous test uses 5 ml of reagent and 50  $\mu$ l of blood sample, whereas the modified O.F.T. uses 200  $\mu$ l and 2  $\mu$ l respectively. Nevertheless, the ratio of sample to reagent is the same as for the previous test i.e. 1 : 101 (figure.1.1.). *Second*, the shape of the bottom tube -

the previous test uses a round bottom tube but the modified O.F.T. uses a sharp bottom tube (figure.1.1.). *Third*, how to read the test - the previous test is read from the side of the tube either by naked eye or a spectrophotometer but the modified O.F.T. test is read from above the test tube by observing the button formation (figure 1.2.).

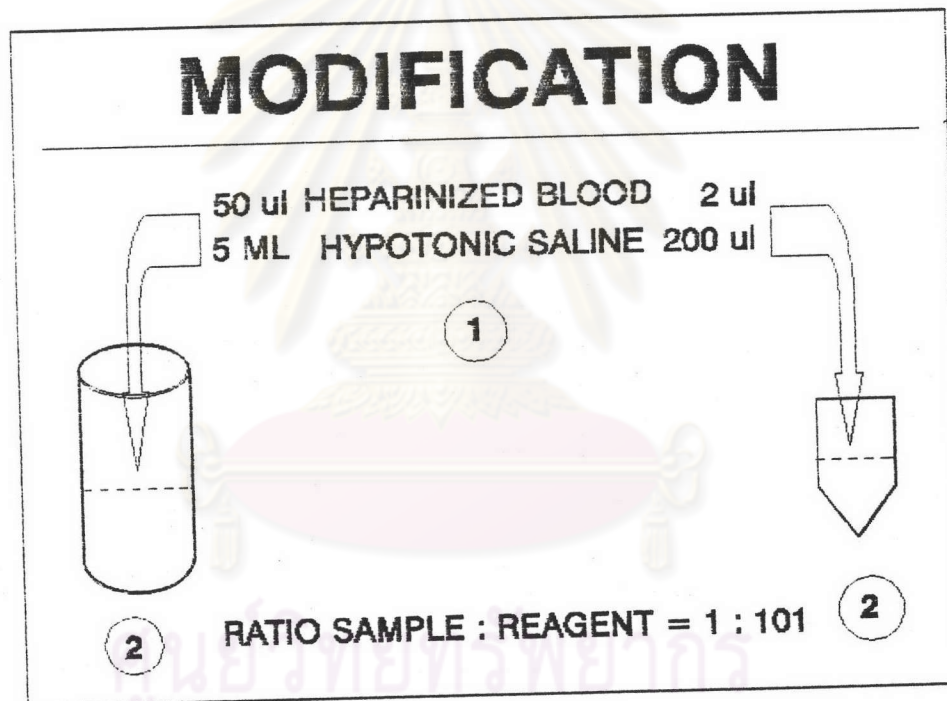


Figure 1.1. The first and second modification of the modified O.F.T. .

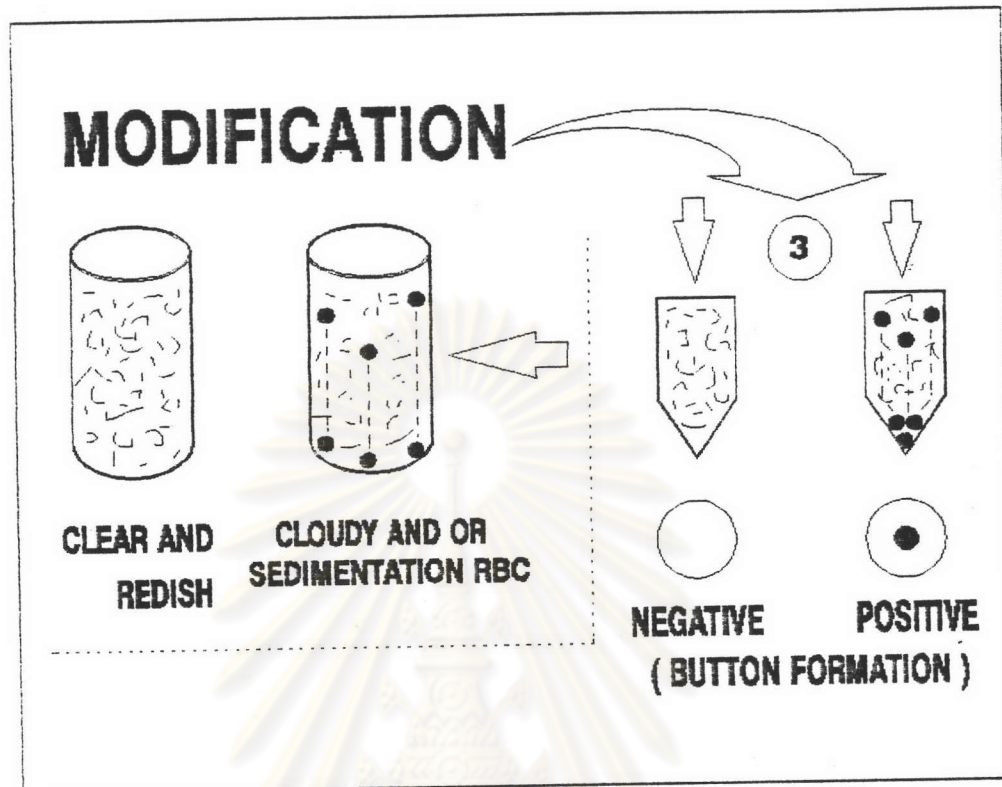


Figure 1.2. The third modification : how to read the test of the modified O.F.T.

#### OPERATIONAL DEFINITION

The button formation is defined as a small bright red dot and has a sharp edge formed by unlied erythrocytes. The test result will be read as positive and interpreted as showing an increased resistance to hypotonic saline solution if the button formation is seen. Those subjects who have positive test may suffer from  $\alpha$  thalassemia trait,  $\beta$  thalassemia trait,  $\beta$  thalassemia major, hemoglobin E trait, hemoglobin E disease and also double heterozygous  $\beta$

thalassemia/hemoglobin E (hemoglobinopathies). And therefore, if the test result is positive we would proceed with further investigations such as hemoglobin electrophoresis and Hb A<sub>2</sub> determination. Conversely, if the test was negative (button formation is not visible) it would be interpreted that the erythrocytes of those subjects have no increased resistance to hypotonic saline solution.

Preliminary study on the modified O.F.T. used a single saline concentration (0.35 %) among 38 subjects consisting of 29 cases of hemoglobinopathies (15  $\beta$  thalassemia trait; 3  $\beta$  thalassemia major; 5 double heterozygous  $\beta$  thalassemia/Hb E and 6 Hb E trait) and 9 normal subjects. The results showed that the test has a sensitivity 96.6 %, specificity 100 %, positive predictive value 100 % and negative predictive value 90 % to detect hemoglobinopathy cases. The test was unable to detect only one out of the six cases of Hb E trait.

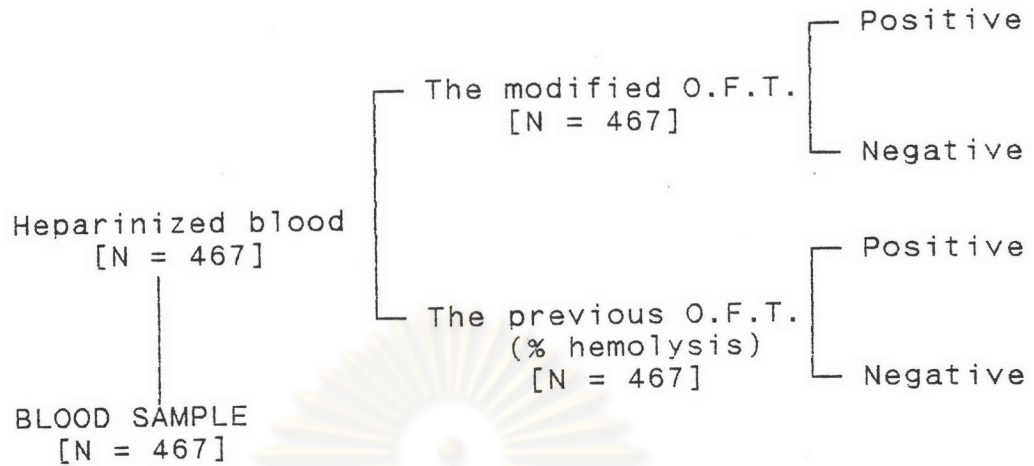
#### CONCEPTUAL FRAMEWORK

In this study, the modified O.F.T. was compared with the previous osmotic method which based on percentage of hemolysis. The comparison was carried out using a single saline concentration : 0.36 %. Then, a standard screening procedure was used as a standard for this comparison in order to detect hemoglobinopathies ( $\alpha$  thalassemia trait,  $\beta$

thalassemia trait, Hb E trait and Hb E disease) among asymptomatic adult people in the general population (see figure 1.3.).



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EDTA Sample : - RBC indices. If MCV < 80 fl ---> serum ferritin determination (use plasma from heparinized blood sample)  
 - Hemoglobin electrophoresis  
 - Hb; Hb A<sub>2</sub> and Hb F determination  
 - Total bilirubin level  
 - RBC morphology

		Standard screening procedure	
		[+] HB PATHY	[-] HB PATHY
MODIFIED O.F.T	[+]		
	[-]		

		Standard screening procedure	
		[+] HB PATHY	[-] HB PATHY
PREVIOUS O.F.T.	[+]		
	[-]		

Figure 1.3. Conceptual framework

## RESEARCH QUESTION

Primary research question : does the modified O.F.T. that uses a single saline concentration of 0.36 % and is visualized by button formation of unlysed erythrocyte have a sensitivity of at least 80 % to screen hemoglobinopathies (α thalassemia trait, β thalassemia trait, Hb E trait and Hb E disease) among asymptomatic adult people in general population when it is compared with a standard screening procedure ?

Secondary research question : which test has a better diagnostic performance to detect hemoglobinopathies (α thalassemia trait, β thalassemia trait, Hb E trait and Hb E disease) , when a comparison is made between the modified O.F.T. and the previous O.F.T. in relation to a standard screening procedure (see appendix 3) ?

## RESEARCH OBJECTIVES

1. To prove that the modified O.F.T visualized by the button formation of unlysed erythrocytes has a good diagnostic performance to detect hemoglobinopathies.
2. To compare the modified and the previous O.F.T.
3. To investigate the factors that may influence test performance of the modified O.F.T.