CHAPTER VI
CONCLUSION

1. To determine the amounts of circulating DNA among pregnancies with and without Hb Bart's hydrops fetalis, TaqMan real time PCR assay was developed for detection of ubiquitous GAPDH gene in maternal plasma DNA. Seventy six pregnancies with no clinical signs of complication during 10-32 weeks of gestation including 21 pregnancies with affected fetus and 55 pregnancies with un-affected fetus were studied. Plasma DNA levels between the two groups were overlapped and were not completely difference although those with Hb Bart's hydrops fetalis trend to be higher elevated than those without Hb Bart's hydrops fetalis.

2. Genotype-phenotype correlation and laboratory diagnostics of five abnormal hemoglobin (Hb) including an α-chain and four β-chain variants were studied. The α-chain variant was the Hb Q-Thailand ($\alpha_2^{74Asp\rightarrow His}\beta_2$) ($-\alpha^{4.2}$) which was found in association with α, β-thalassemia and Hb E. At least two Hb derivatives were observed for individuals with this Hb variant, the Hb Q-Thailand ($\alpha^{QT}{}_2\beta^{A}{}_2$) and the Hb QA_2 ($\alpha^{QT}{}_2\delta_2$), which were detected by HPLC and capillary electrophoresis system. In addition, Hb QE ($\alpha^{QT}{}_2\beta^{E}{}_2$) derivative was detected in double heterozygote for Hb QT/Hb E syndrome. α-Globin gene haplotype analysis indicates a single origin of Hb Q-Thailand in Thai population. Four β-chain variants include Hb Hope ($\alpha_2\beta_2^{136Gly\rightarrow Asp}$), Hb Tak ($\alpha_2\beta_2^{147Term\rightarrow Thr}$), Hb Korle-Bu ($\alpha_2\beta_2^{73Asp\rightarrow Asn}$) and Hb Phimai ($\alpha_2\beta_2^{72Ser\rightarrow Thr}$). As for Hb Q-Thailand, Hb Hope was found in association with α & β-thalassemia and Hb E. Patients with Hb Hope/Hb H-disease had minute amounts of Hb Bart's but not Hb H. In a compound Hb Hope/β^0-thalassemia it was found that Hb A_2 was still at the diagnostic range of β-thalassemia carrier. β-Globin gene haplotype analysis points to a single origin of Hb Hope in Thai population. Hb Tak is a β-globin chain variant with high oxygen affinity. In this studied, the secondary erythrocytosis associated with heterozygous Hb Tak/$\delta\beta^0$-thalassemia was reported for the first time. This condition had similar phenotype with that of homozygous Hb Tak / α^+-

thalassemia encountered. Next, a complex interaction of Hb Korle-Bu / Hb E and α^0-thalassemia found in a pregnant Thai woman with mild hypochromic microcytic anemia was studied. The genotype-phenotype interaction was compared with her family members with other related genotypes. The last Hb variant identified was the Hb Phimai [β72(E16)Ser→Thr], a novel β-chain variant found in another pregnant woman who also carried Hb Constant Spring. This variant was caused by a AGT-ACT transversion at codon 72 which involved in the formation of heme pocket of the Hb molecule. It is speculated that Hb Phimai is unstable and might have high oxygen affinity. Its HPLC and capillary electrophoresis patterns were similar to that of the Hb Hope, also found in Thai population. A multiplex allele specific PCR for differential diagnosis of these two Hbs was therefore developed. The characterization methods used and the data obtained in this study will prove useful for laboratory diagnosis and study of hemoglobinopathies in Thailand and should also facilitate the prevention and control program of hemoglobin disorders in Thailand.

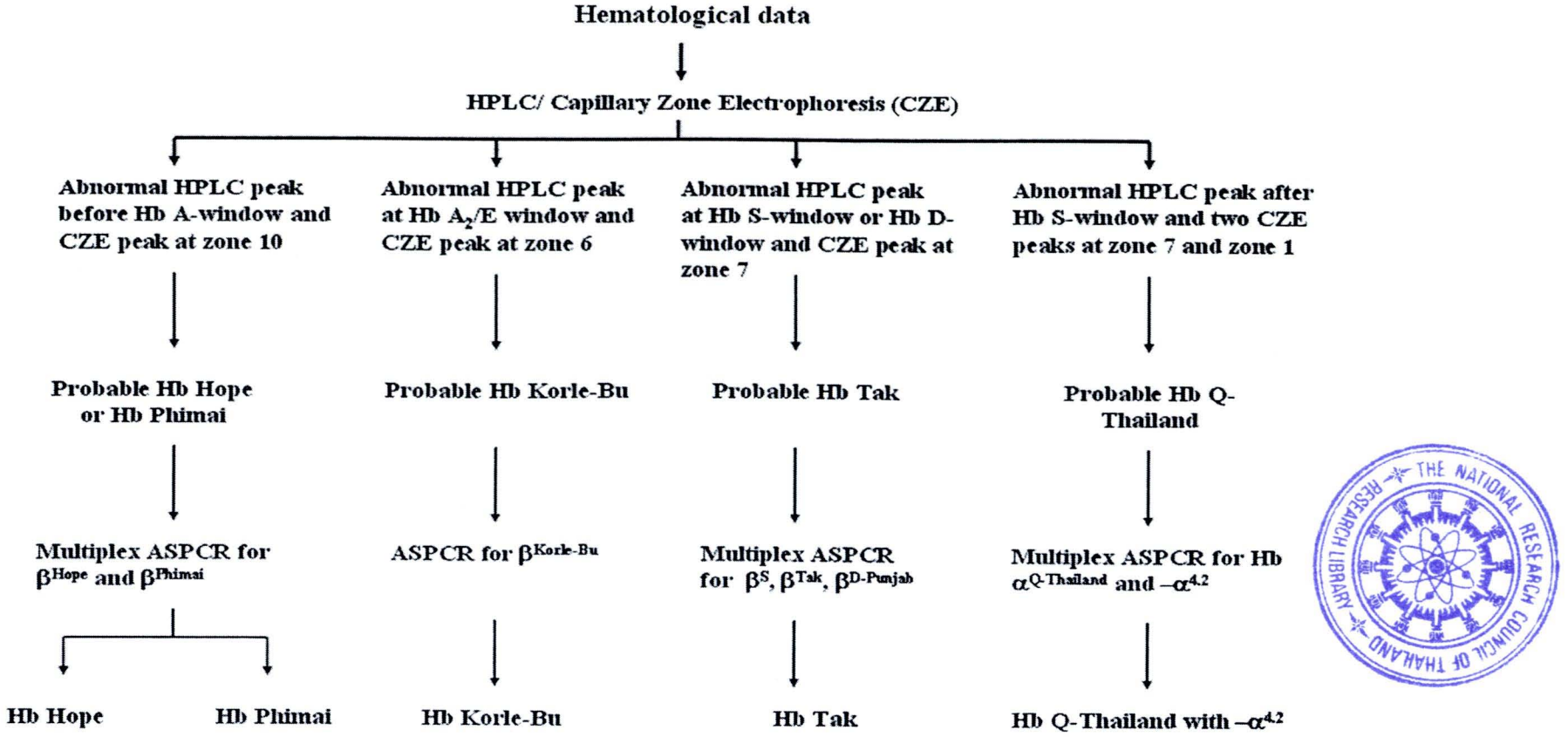

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Figure 23 A schematic diagram for Hb and DNA characterizations of Hb Hope, Hb Phimai, Hb Korle-Bu, Hb Tak and Hb Q-Thailand developed in this study.