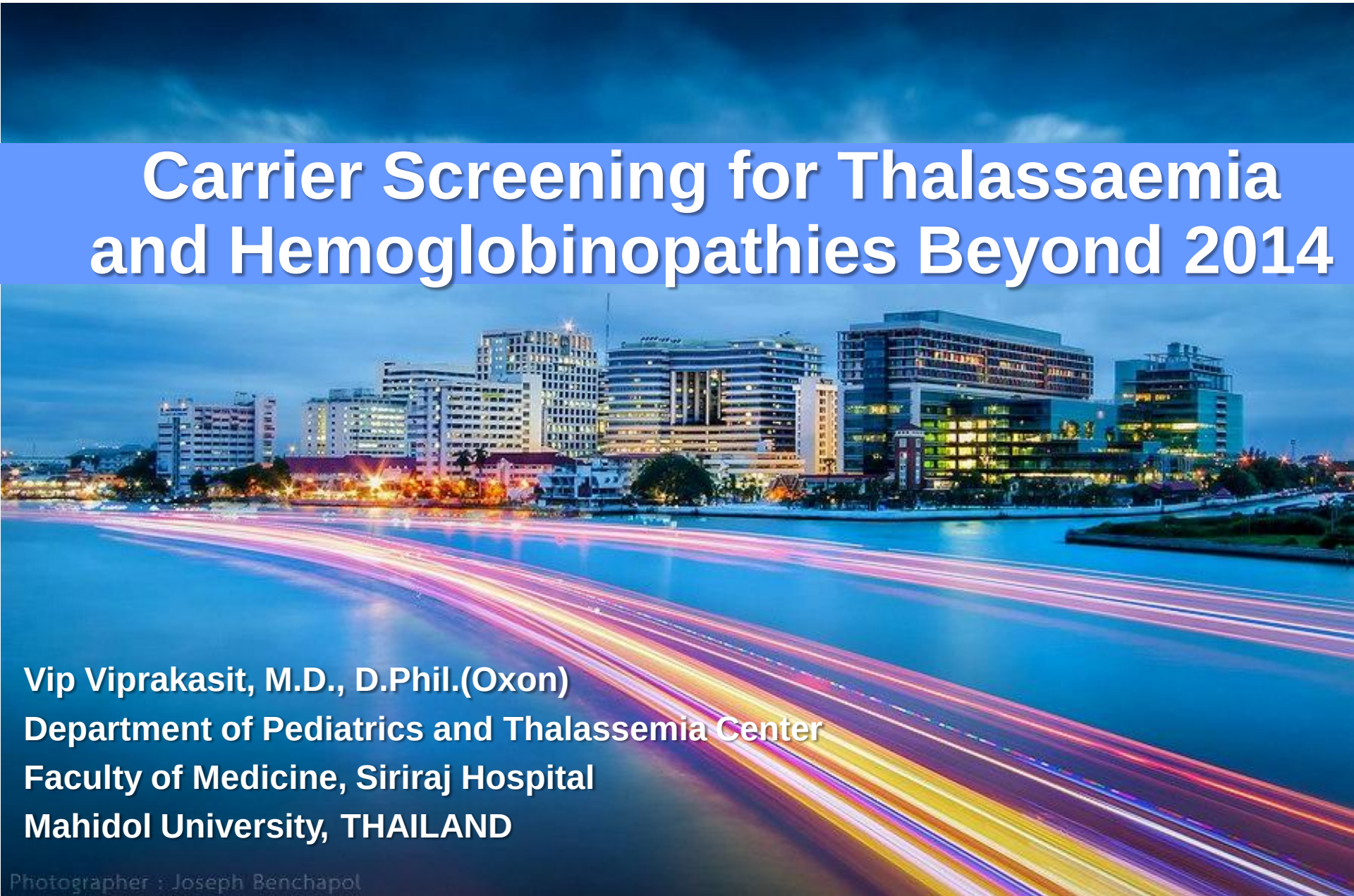
Carrier Screening for Thalassaemia and Hemoglobinopathies Beyond 2014



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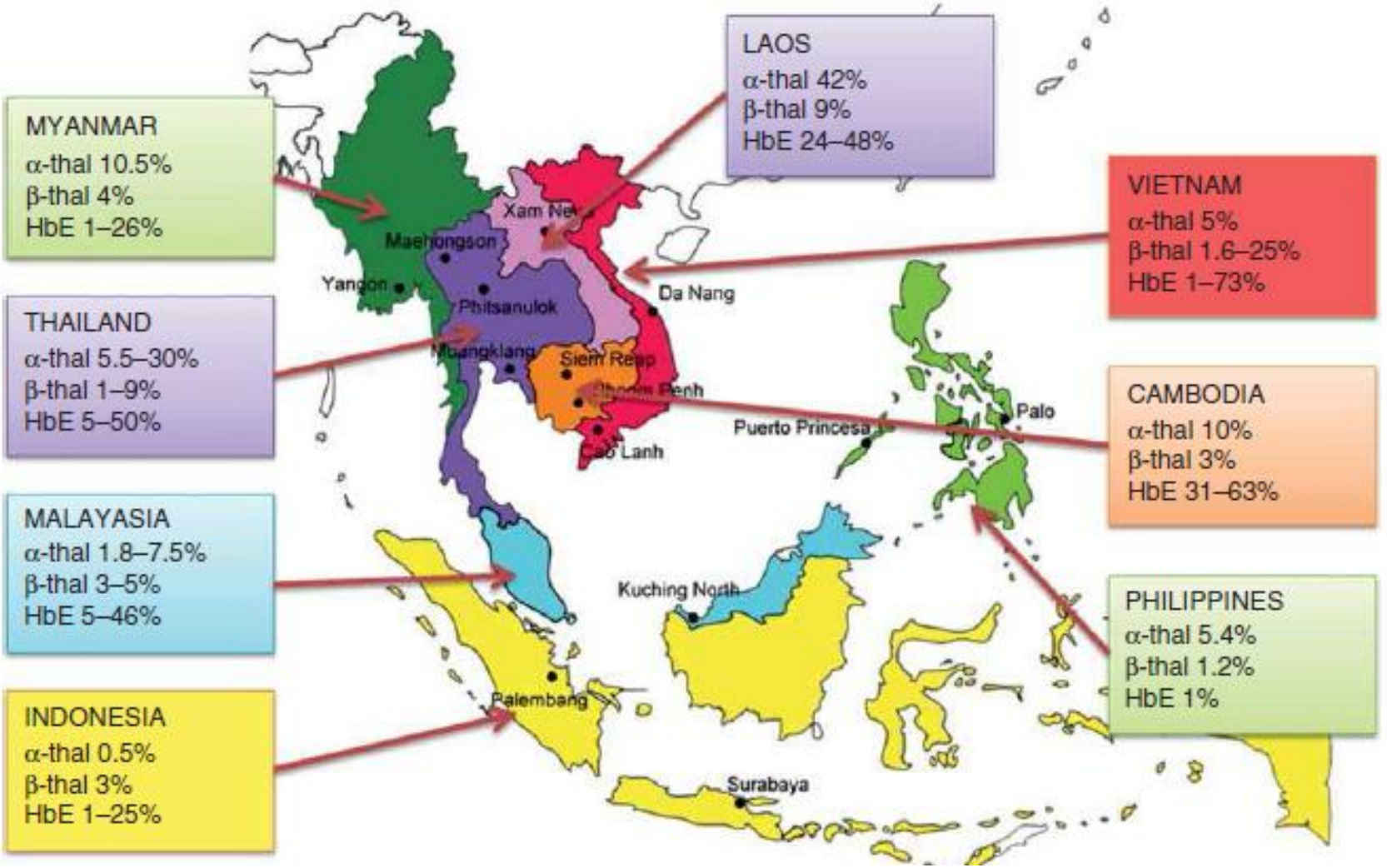
Department of Pediatrics and Thalassemia Center
Faculty of Medicine, Siriraj Hospital
Mahidol University, THAILAND

Photographer : Joseph Benchapol

Scope of my talk

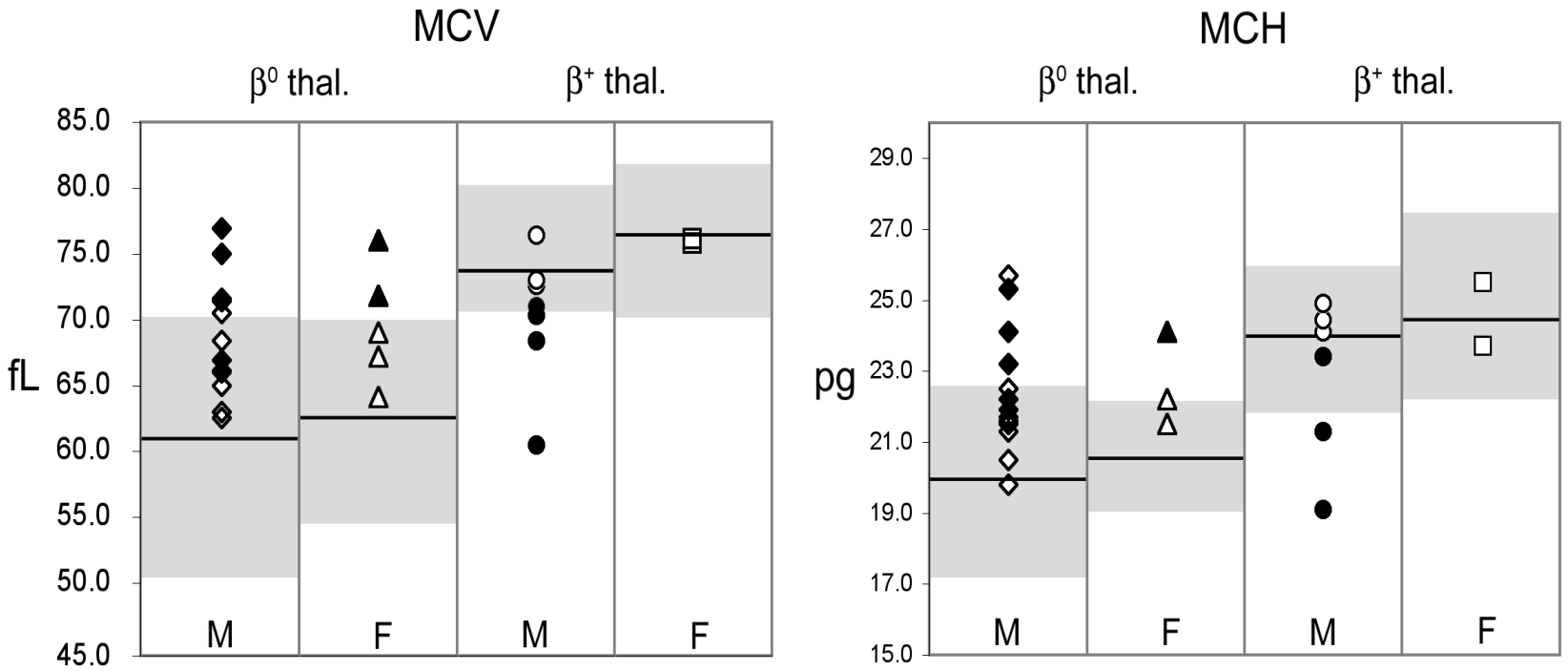
- Why do we need a new model for thalassemia carrier screening
- Pre-marital & reproductive screening
- Newborn screening
- Future plan

Prevalence of thalassaemia and hemoglobinopathy in South East Asian countries



Viprakasit V et al. *Expert Opinion on Orphan Drug 2014*

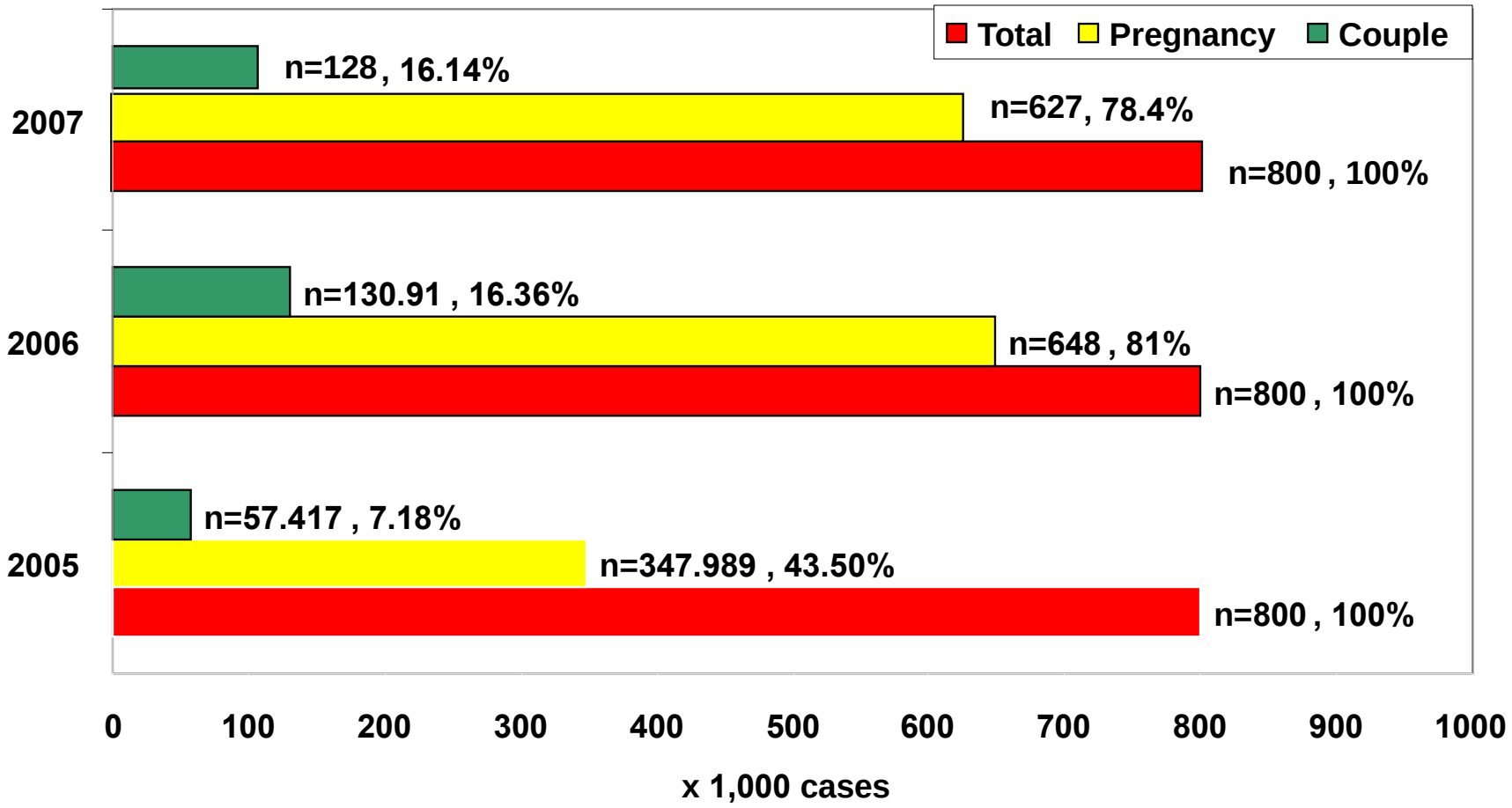
The scatter plots of MCV and MCH in individuals with β thalassemia with and without α thalassemia



Shaded boxes represent upper-lower range of values in β thalassemia trait with normal α genes (αα/αα) with the black lines denote the average (X) in each group. The black symbols represent individual cases with β thalassemia traits and **α^0 thalassemia** while the white symbols signify β thalassemia traits with **α^+ thalassemia.**

Viprakasit V *et al*. *CCLM 2013*

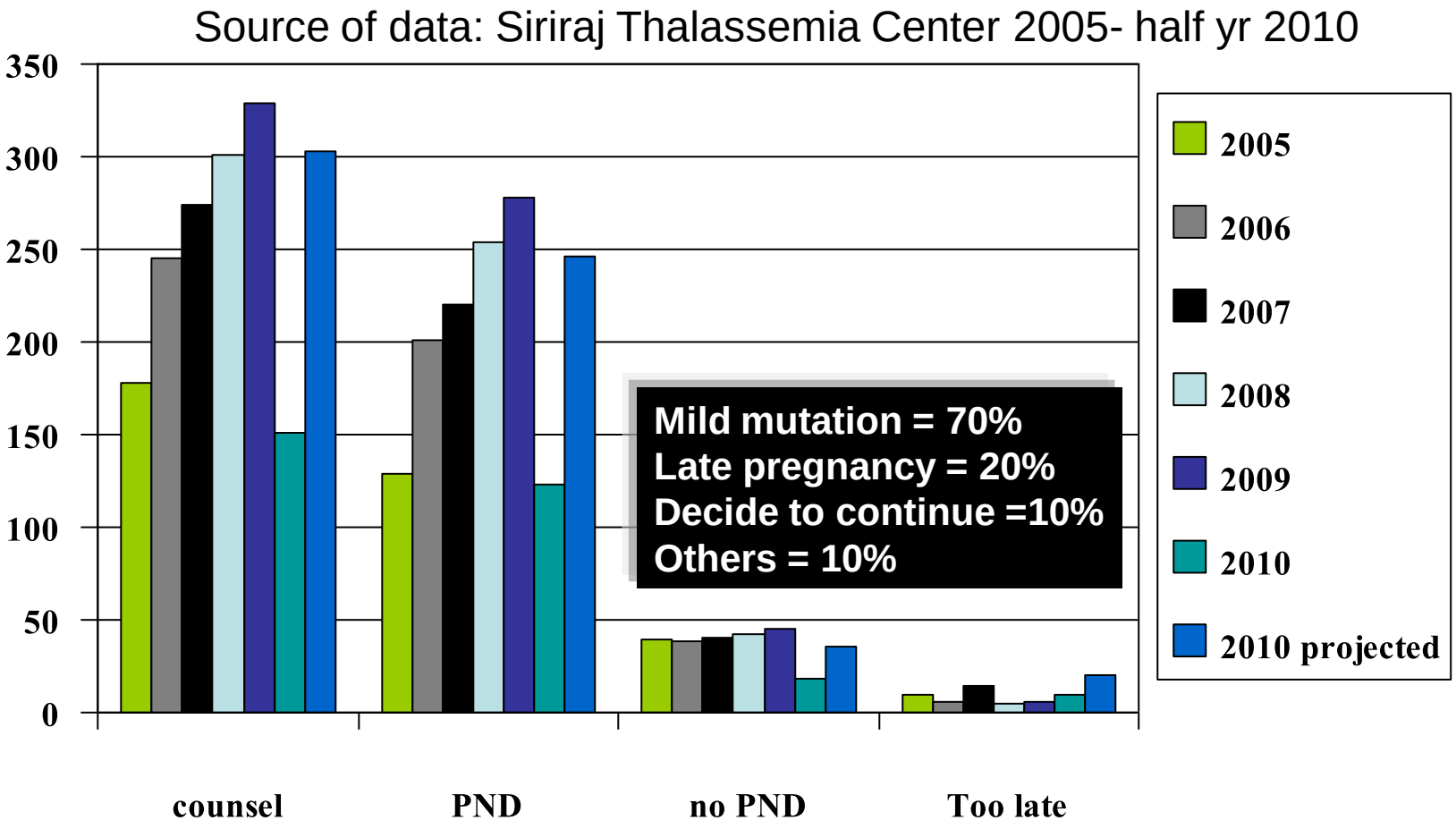
Current obstacle in prevention and control program for severe thalassemia in Thailand



Reported by Bureau of Health Promotion, Department of Health

The 13th Thailand National Thalassemia Conference 2007

Siriraj Hospital ’s experience on couples at risk’ decision regarding PND counseling



Vip Viprakasit & Chanin Limwongse, unpublished data 2012

Although the number of the participant couples was on the rise, detected fetus with severe thalassemia syndrome was **very low (<20%)**

Logical steps of prevention & control program for severe thalassemia

- Identification of disease carrier in a population
- Education on them on thalassemia/disease o
- Dissuasion of marriage between c
- Alternative options o pring in couple at risk: adoption egg donation and no offspring
- Pre tion genetic diagnosis
- Prenatal diagnosis and counseling-Termination of pregnancy

When and Whom to start the program ???

What to concern when you start to screen for thalassemia carriers

- Screening program should be supported by public education and regulatory structures
- All medical personnel should empower individuals to make informed decision
- We must ensure that people are protected against discrimination as a result of their test

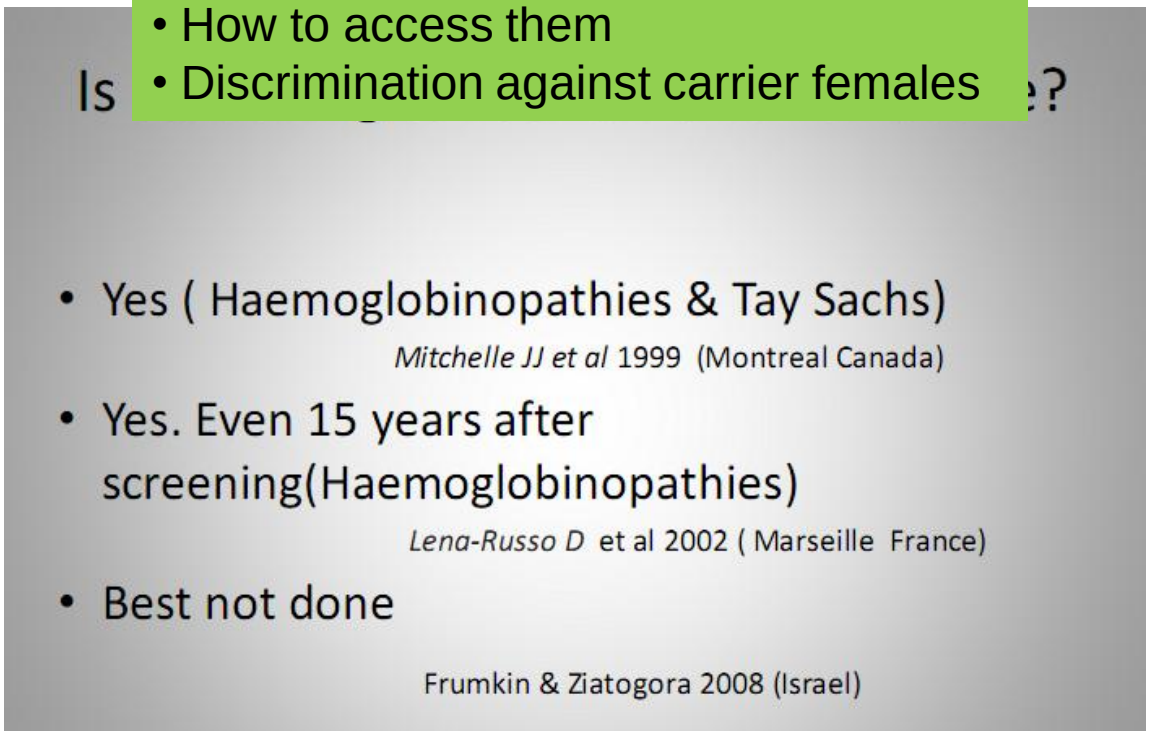
“Whom” should be screened…

- Cascade screening
- Antenatal screening
- Premarital screening
- School children

Cascade screening:

Antenatal screening:
- Must have PBD to offer

Premaritall screening:
- How to access them
- Discrimination against carrier females

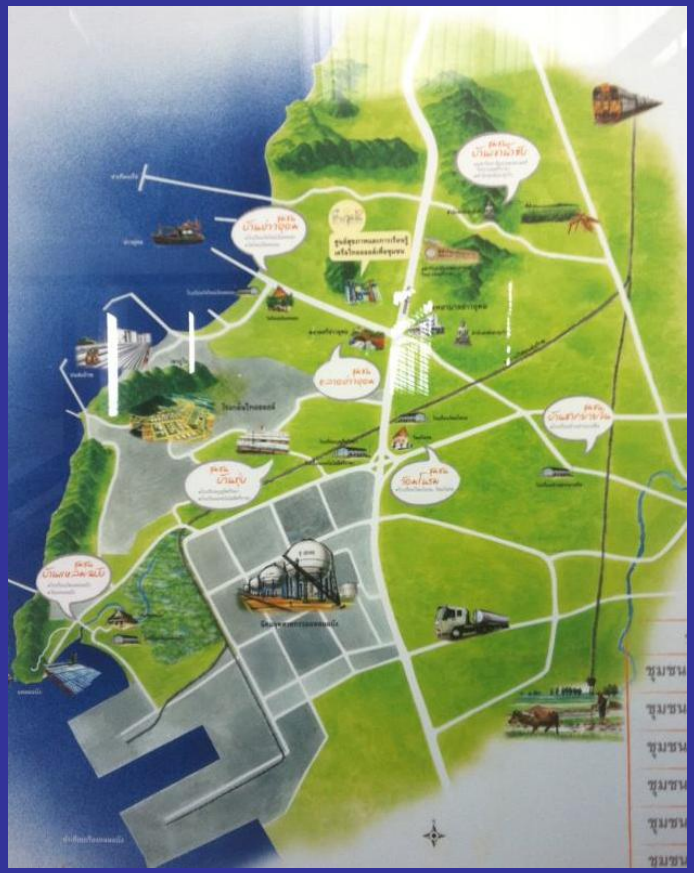


Our Future Paradigm Shift: Udom Bay Project

A new model for Thal Screening

My "Framingham Hb Study" at Auwudom project

Start in 2012



Methods

Population at reproductive age (10-50) at Laemchabang, Sriracha district, Chonburi
N=1000

Standard thal. screening
MCV + DCIP

New screening program
CBC + Hb typing
+ α^0-thal DNA

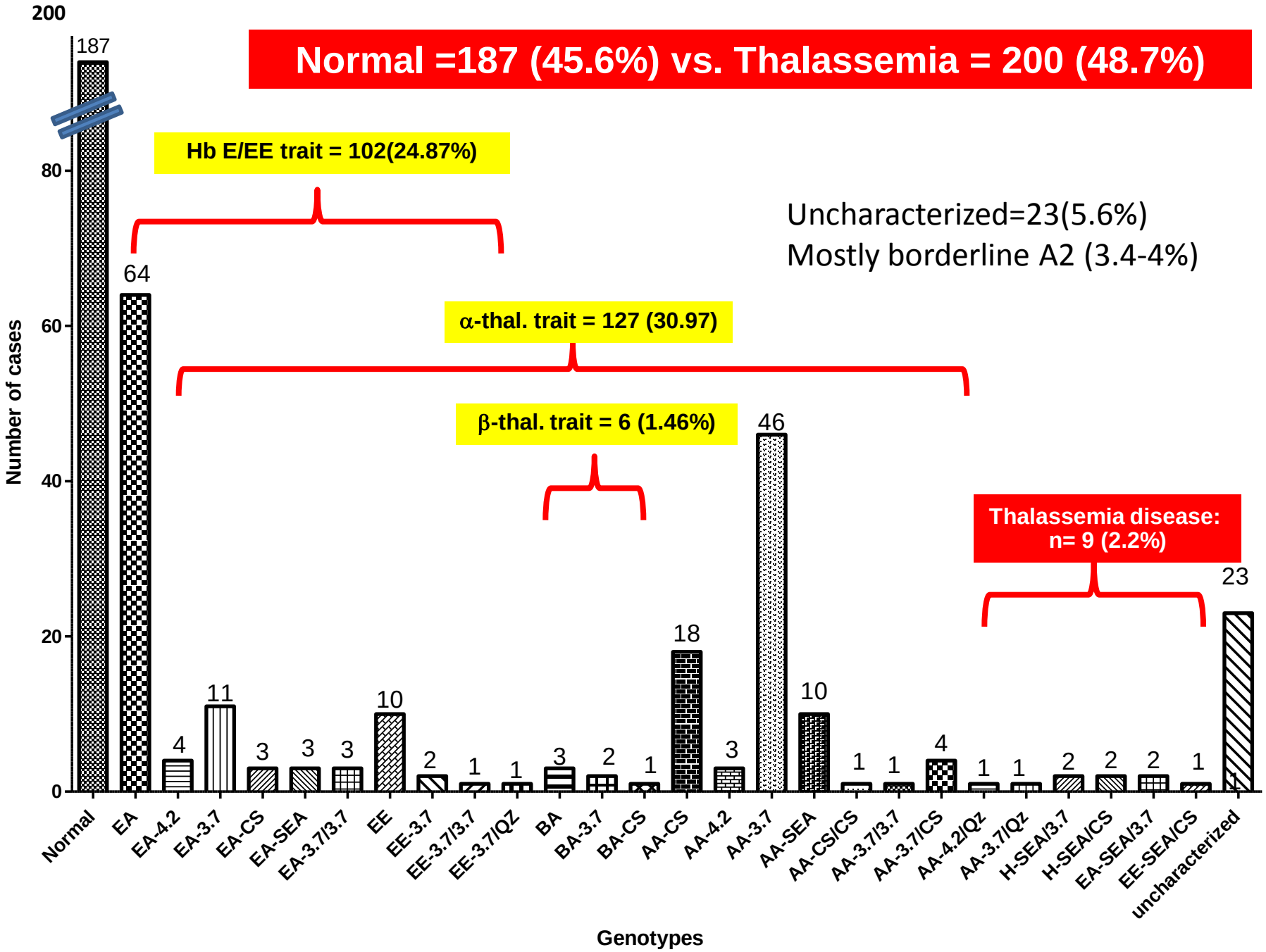
Comprehensive iron study :
-Serum iron
- TIBC
- Serum ferritin
- Transferrin sat.
- NTBI
- LPI
- Hepcidin

Comprehensive hemoglobin diagnosis:
-DNA diagnosis for 13 common α-thal mutations*
- DNA diagnosis for 8 common β-thal mutations#
- MLPA and direct DNA seqencing

Thalassemia
vs.
Normal

* = $--^{SEA}$, $--^{THAI}$, $--^{FIL}$, $--^{MED}$, $-\alpha^{20.5}$, $-\alpha^{3.7}$, $-\alpha^{4.2}$ CD59, Hb Constant Spring, Hb Pakse, CD30, initiation codon, Hb Quong Sze.

= -28, CD8/9(+G), CD17(A-T), IVSI-I(G-T), IVSI-5,(G-C), CD26(HbE), CD41/42(-TCTT), CD71/72(A+) , IVSII-654(G-T), 105bp, 619bp, 3.5kb, SEA HPFH, Filipino, Hb Lepore, Asian Indian deletions inversion, Chinese, HPFH-6, Siriraj-thalassemia.


Normal =187 (45.6%) vs. Thalassemia = 200 (48.7%)
Hb E/EE trait = 102(24.87%)
α-thal. trait = 127 (30.97)
β-thal. trait = 6 (1.46%)
Thalassemia disease: n= 9 (2.2%)
Uncharacterized=23(5.6%)
Mostly borderline A2 (3.4-4%)
Number of cases
Genotypes
200
80
60
40
20
0
187
64
4
11
3
3
3
10
2
1
1
3
2
1
18
3
46
10
1
1
4
1
1
2
2
2
1
23
Normal
EA
EA-4.2
EA-3.7
EA-CS
EA-SEA
EA-3.7/3.7
EE
EE-3.7
EE-3.7/3.7
EE-3.7/QZ
BA
BA-3.7
BA-CS
AA-CS
AA-4.2
AA-3.7
AA-SEA
AA-CS/CS
AA-3.7/3.7
AA-3.7/CS
AA-4.2/QZ
AA-3.7/QZ
H-SEA/3.7
H-SEA/CS
EA-SEA/3.7
EE-SEA/CS
uncharacterized


Newborn Screening:
Because you touch the future everyday

If Untreated, Disorders

- Can result in:
  - Growth problems
  - Developmental delays
  - Behavioral/emotional problems
  - Deafness or blindness
  - Retardation
  - Seizures
  - Coma, sometimes leading to death

CH & PKU

Newborn Screening (cord blood)

Can we diagnose thalassaemia at birth

Back to the "Future"

WHY Newborn Screening ?

1.Newborn screening Program has been in existence but only for prevention and control of mental retardation.

2.The good period for Hb Bart's detection in α-thalassemia diagnosis.
> 1 year of age can be detected only by DNA analysis.

3.Detection of Hb E at birth.

Newborn screening for Thalassemia and hemoglobinophathies

▶ Methodology :

High performance liquid chromatography (HPLC)
Isoelectric focusing (IEF)

▶Advantage of IEF beyond HPLC method :

Can be used for both screening and confirmation.
Applicable for newborn screening specimens (DBS).
Efficiency of cost for screening.
High resolution for Hb variants detection.

The Registry and Surveillance System for Hemoglobinopathies or RuSH,

- The National Heart, Lung, and Blood Institute (NHLBI)/NIH and the Division of Blood Disorders (DBD) at CDC develop a state-based monitoring system for SCD and thalassemia to identify and gather information on all people living with a hemoglobinopathy diagnosis of sickle cell diseases or thalassemia

- The participating states (California, Florida, Georgia, Michigan, North Carolina, Pennsylvania, and New York)

- during 2004-2008.

Genetics inMedicine
Official Journal of the American College of Medical Genetics and Genomics

ARTICLE PREVIEW

GENETICS IN MEDICINE | ORIGINAL RESEARCH ARTICLE

State-based surveillance for selected hemoglobinopathies

Mary M. Hulihan MPH, Lisa Feuchtbaum DrPH, Lanetta Jordan MD, Russell S. Kirby PhD, Angela Snyder PhD, William Young PhD, Yvonne Greene MPH, Joseph Telfair DrPH, Ying Wang PhD, William Cramer MPH, Ellen M. Werner PhD, Kristy Kenney MPH, Melissa Creary MPH & Althea M. Grant PhD

Affiliations | **Corresponding author**

Most read

Managing incidental findings and research

The Registry and Surveillance System for Hemoglobinopathies or RuSH,

- A pilot program that supported the development of the infrastructure and data collection methods for a state-based surveillance system for selected hemoglobinopathies.
- Total **31,144** individuals who had a hemoglobinopathy diagnosis during the study period were identified in California; 39,633 in Florida; 20,815 in Georgia; 12,680 in Michigan; 34,853 in New York, and 8,696 in North Carolina.
- State health department employees, healthcare providers, academic institutions, community organizations, patients, and families were all important contributors to the program, and they worked closely with each other throughout the entire process.

Original Article

The Association for Clinical Biochemistry & Laboratory Medicine

Annals of Clinical Biochemistry
0(0) 1–11

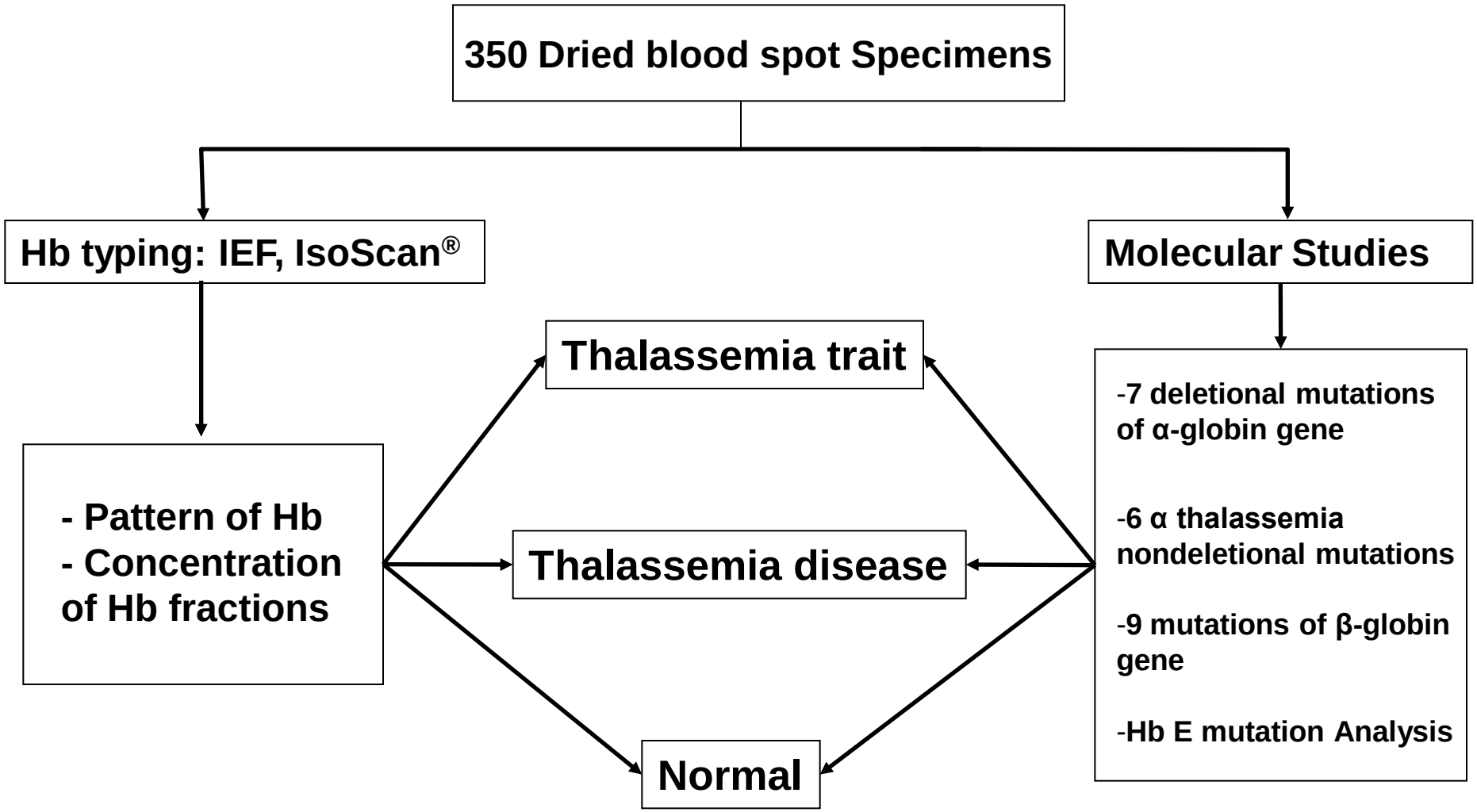
DOI: 10.1177/0004563213491078
acb.sagepub.com
SAGE

Diagnostic applications of newborn screening for α-thalassaemias, haemoglobins E and H disorders using isoelectric focusing on dry blood spots

Punyanuch Jindatanmanusan[1,2], Suchada Riolueang[3,4], Waraporn Glomglao[5], Yaowapa Sukontharangsri[6], Sangkae Chamnanvanakij[1], Kitti Torcharus[7] and Vip Viprakasit[4,5]

Pronpanich, P et al. *Ann Clin Biochem* 2013

Schematic Diagram of Research Planning



Objectives

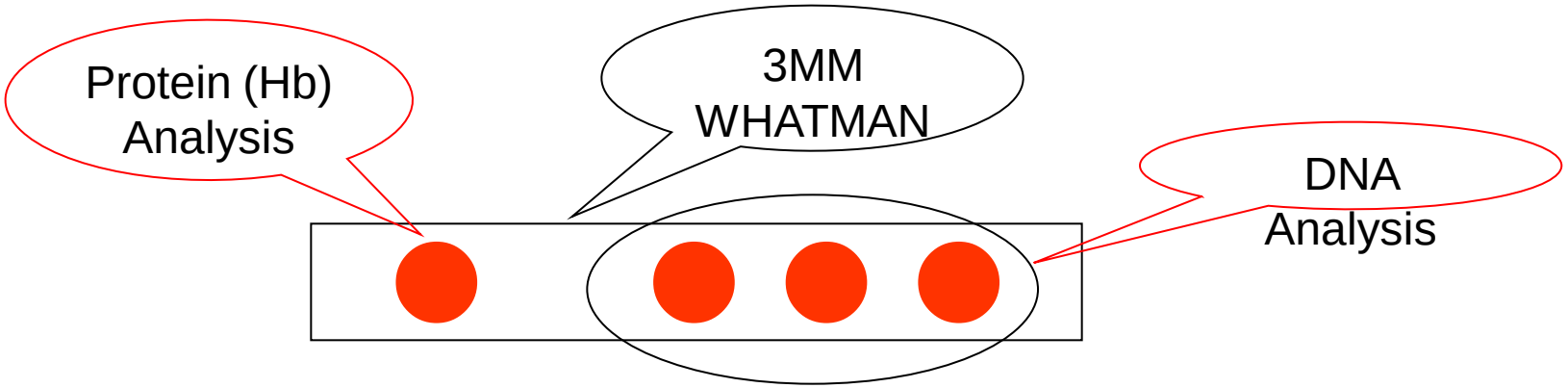
1. To set up a standard range of each hemoglobin profile identified at newborn period for definitive diagnosis for thalassemia diseases and carriers.

2. To determine the sensitivity and specificity of IEF for thalassemia diseases and carriers identification in the newborns in Thailand.

Materials and methods

Subjects

350 dried blood specimens of newborn infants from newborn unit, Pediatrics, Phramongutklao hospital, Bangkok, Thailand.

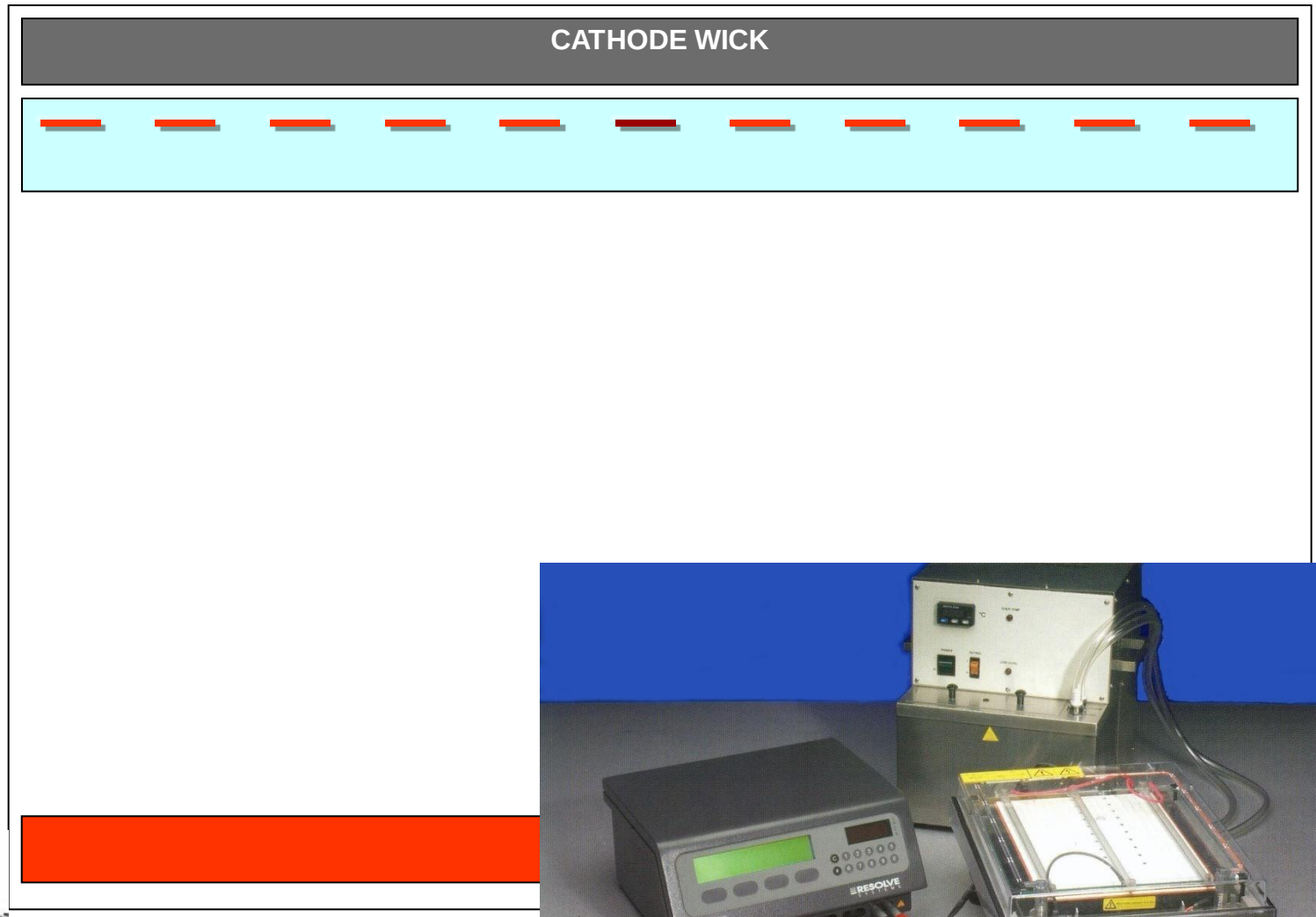


METHOD

Protein (Hb) Analysis

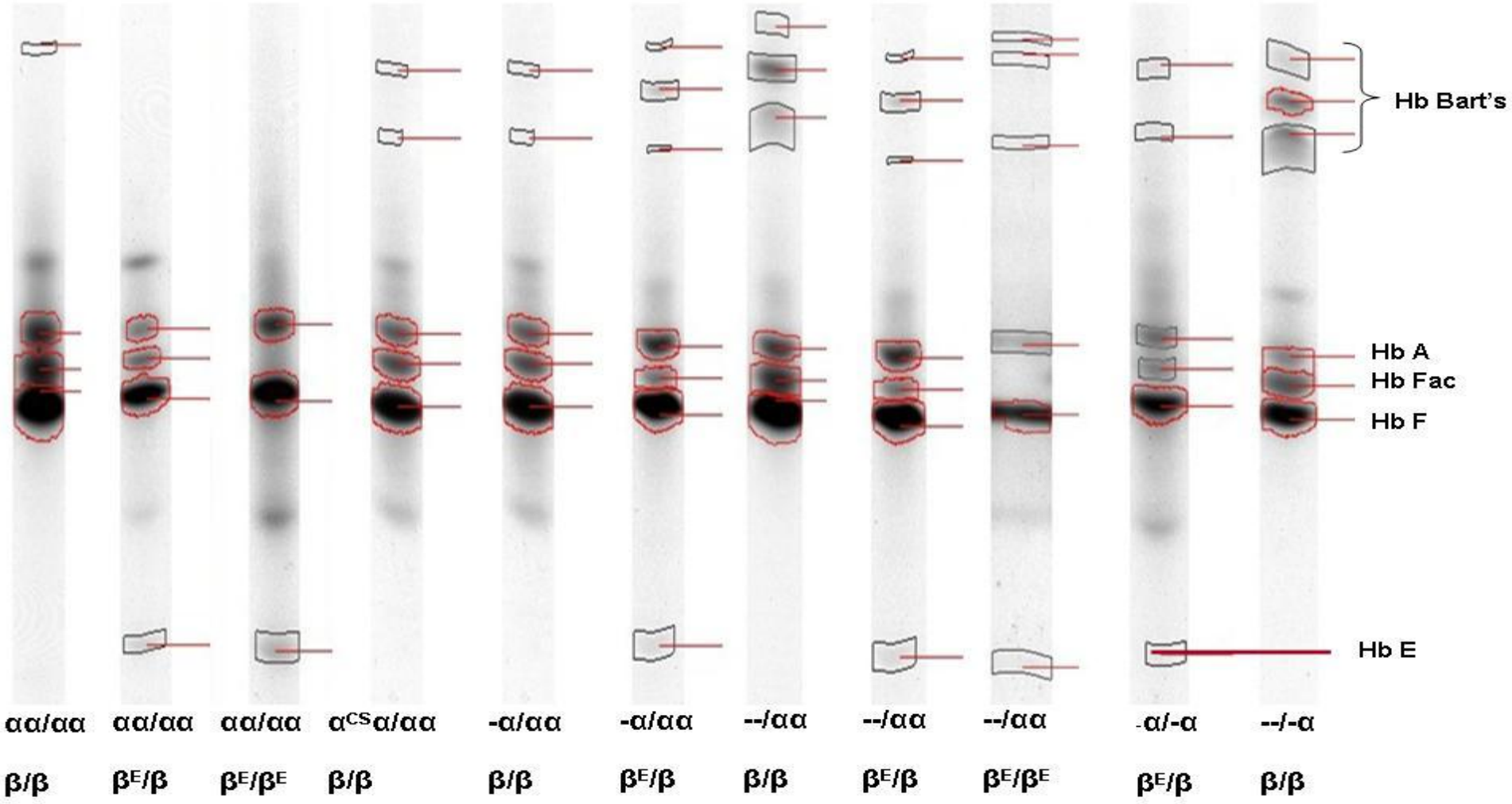
6 mm Ø 2 dots

25 µL Hb Elution solution

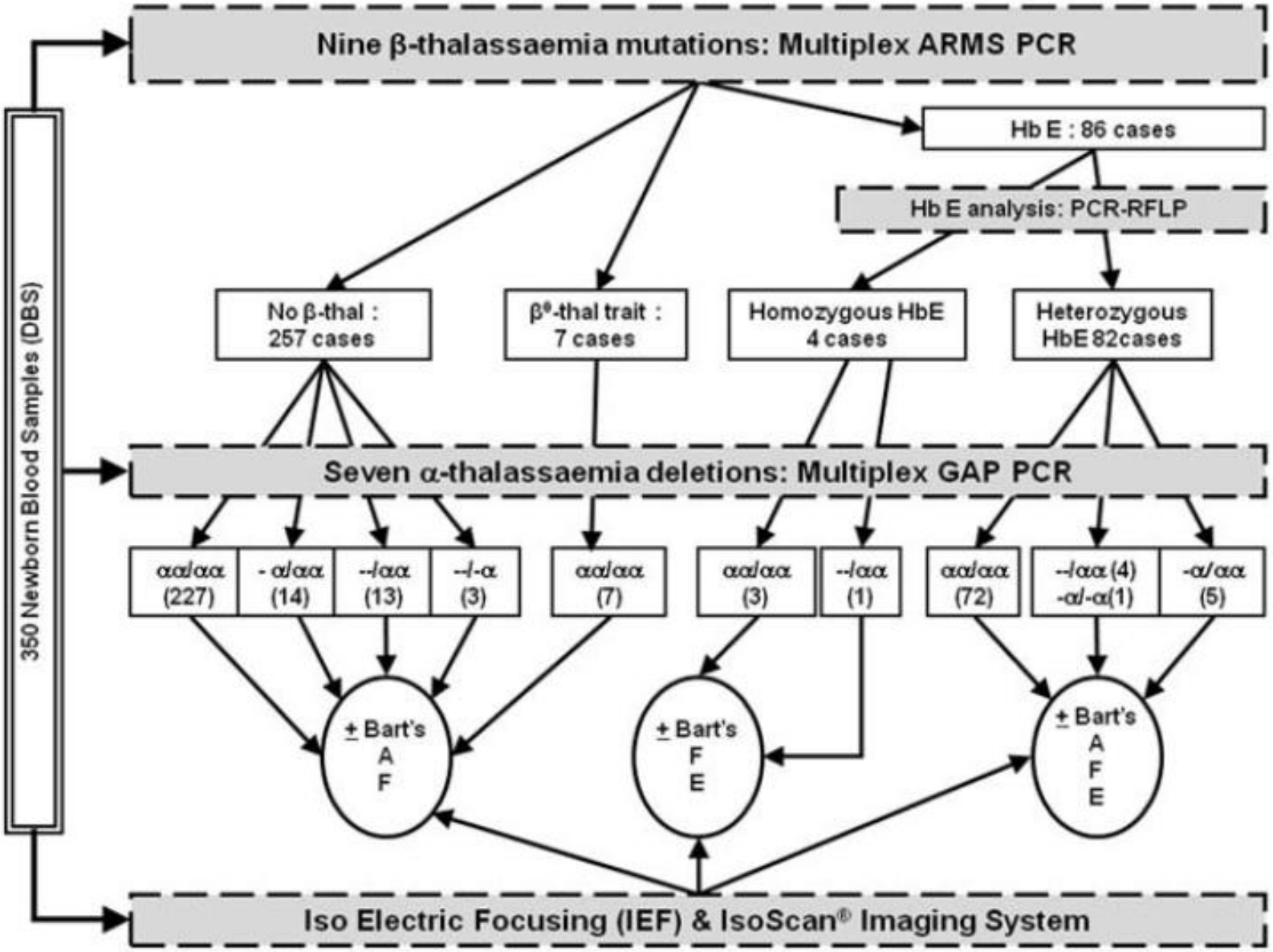


PerkinElmer instruments

IEF for Neonatal Screening using dry blood spots



Pronpanich, P et al. *Ann Clin Biochem* 2013

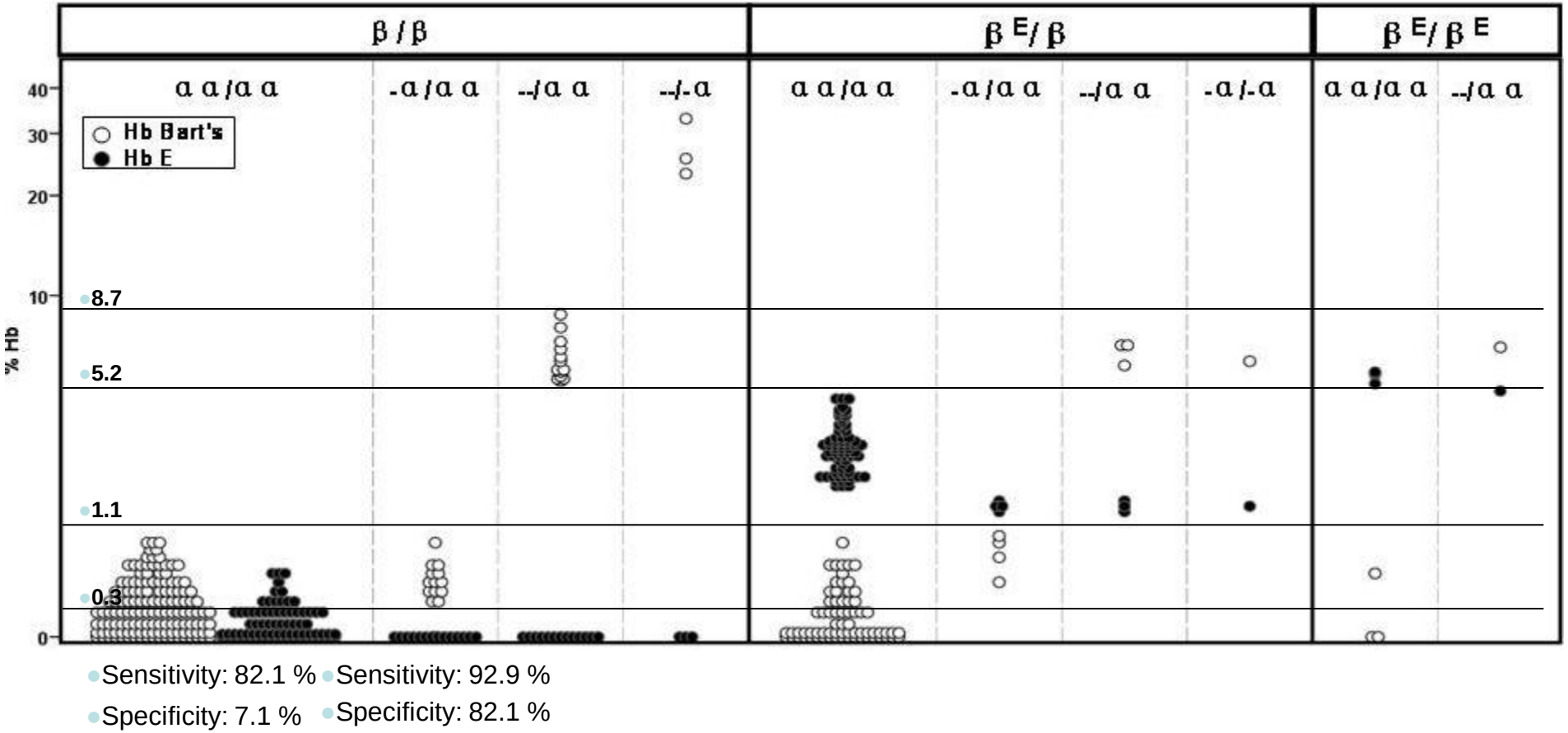


Pronpanich, P et al. *Ann Clin Biochem* 2013

Sensitivity and specificity of IEF for thalassemia diseases and carriers identification in the newborns in Thailand

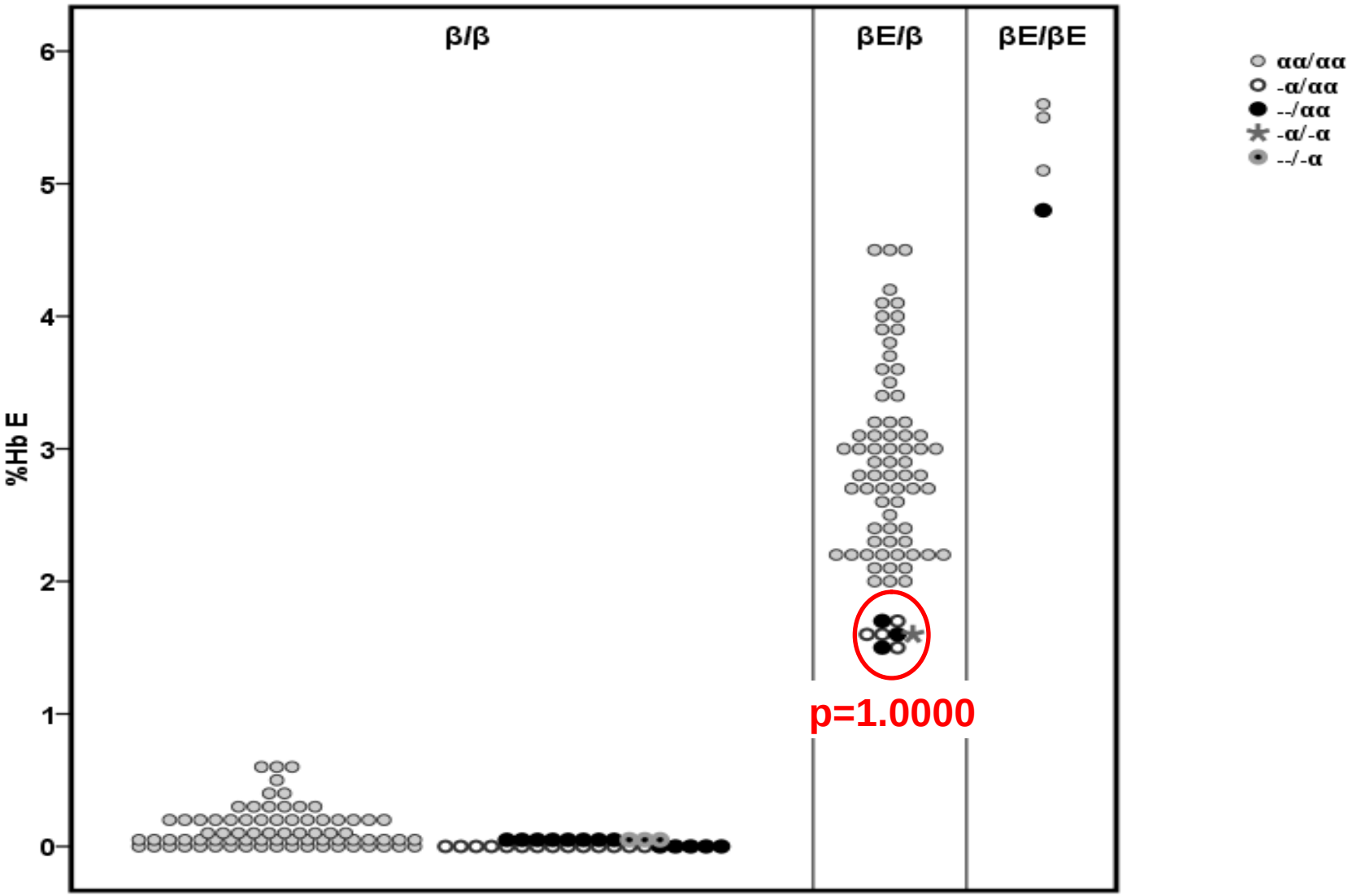
Sensitivity: 100 %

Specificity: 100%

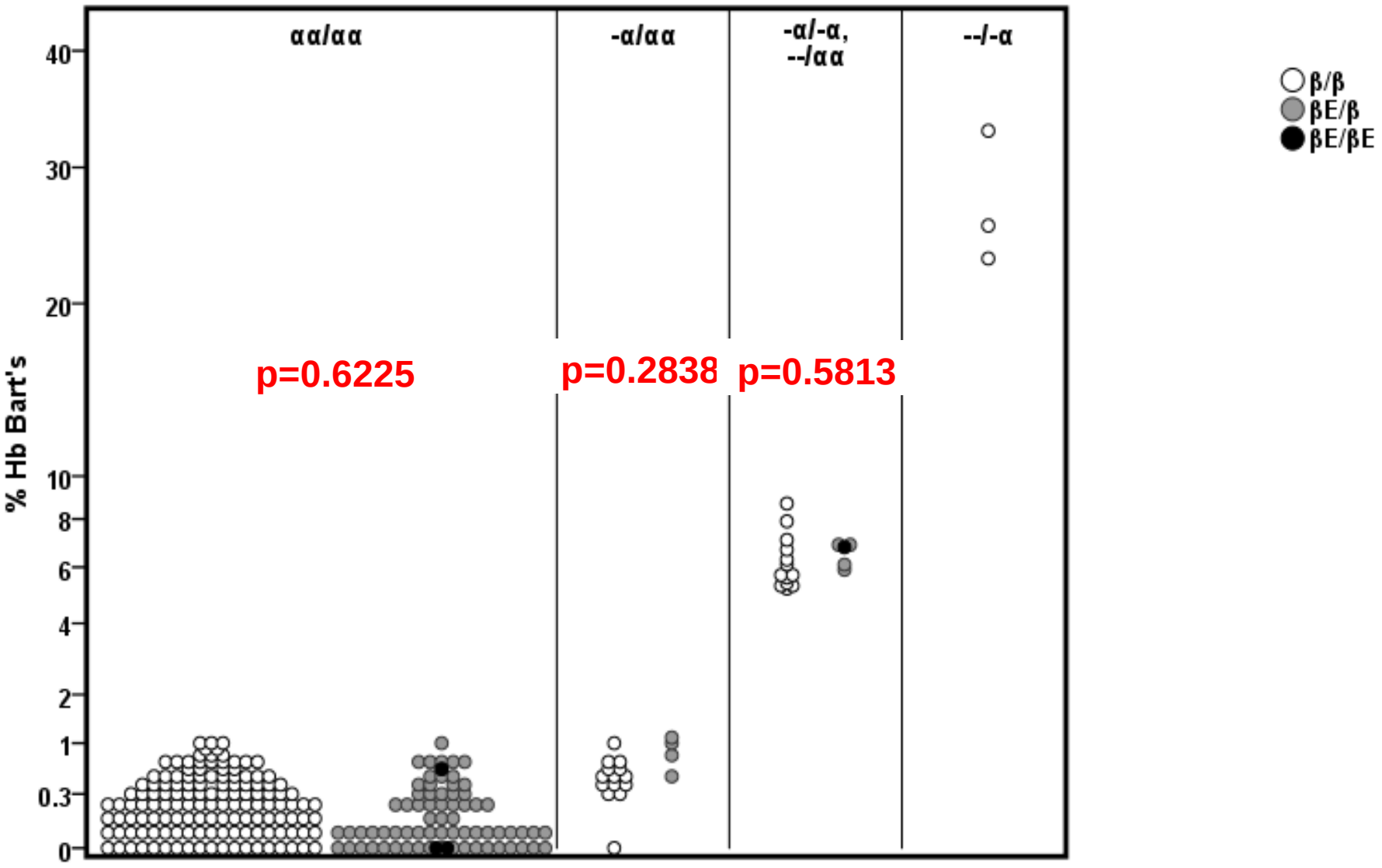


Pronpanich, P et al. *Ann Clin Biochem* 2013

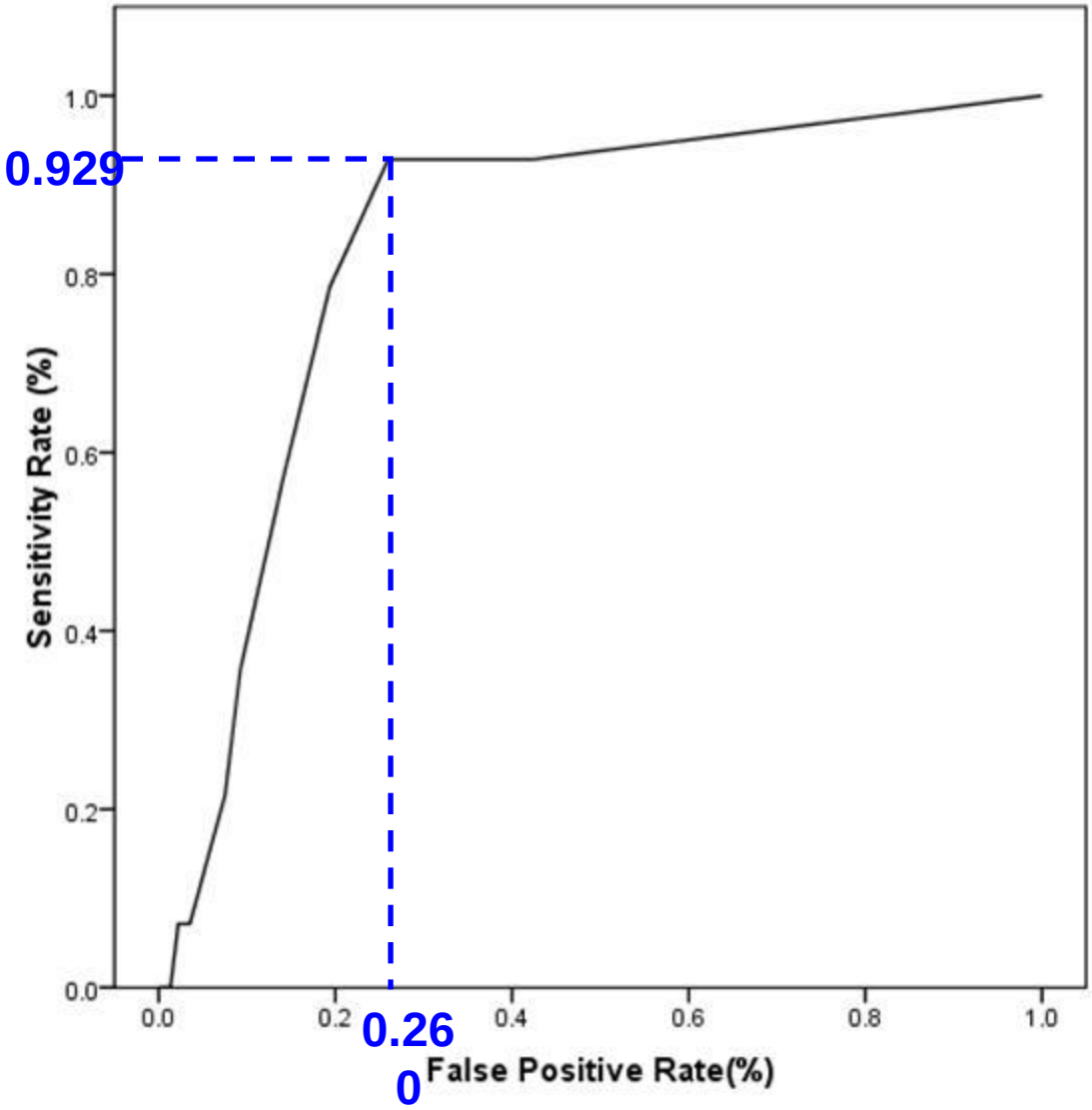
The effect of α-globin gene deletion to Hb E levels



The effect of Hb E mutation to the amount of Hb Bart's



Cut off level for heterozygous α-thalassemia2 diagnosis



Coordinates of the Curve		
Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity
-1.00	1.000	1.000
0.05	0.929	0.423
0.15	0.929	0.344
0.25	0.929	0.260
0.35	0.786	0.194
0.45	0.571	0.141
0.55	0.357	0.093
0.65	0.214	0.075
0.75	0.071	0.035
0.85	0.071	0.022
0.95	0.000	0.013
2.00	0.000	0.000

ROC curves for the diagnosis of heterozygous α -thalassemia-2, based on Hb Barts levels. (Area under curve 0.835).

Neonatal screening with IEF can diagnose Hb E, EE & β^0 thal/Hb E $\pm$ α thalassemia

Table 4 Standard reference range for presumptive diagnosis of α-thalassaemia traits, Hb H and Hb E disorders using the concentrations of Hb Bart's and Hb E.

Hb E(A2) \ Hb Bart's	0–0.25	0.25–1.1	5.2–8.7	23.0–33.0	Interpretation
0–1.0	αα/αα, β^A/β^A	-α/αα, β^A/β^A	--/αα, β^A/β^A	--/-α, β^A/β^A	Genotypes
	Normal	α^+ thal trait	α^0thal trait	Hb H dis.	Phenotypes
1.5–1.7		-α /αα, β^E/β^A	--/αα, β^E/β^A -α/-α, β^E/β^A	--/-α, β^E/β^A	Genotypes
		Hb E and α^+ thal trait	Hb E and α^0 thal trait	Hb E trait and Hb H dis	Phenotypes
2.0–4.5	αα/αα, β^E/β^A				Genotypes
	Hb E trait				Phenotypes
4.8–5.6	αα/αα, β^E/β^E	-α/αα, β^E/β^E	--/αα, β^E/β^E	--/-α, β^E/β^E	Genotypes
	Homo E	Homo E and α^+ thal trait	Homo E and α^0 thal trait	Homo E and Hb H dis.**	Phenotypes

*Known as Hb AE Bart's disease.
**Known as Hb EF Bart's disease.

Pronpanich, P et al. *Ann Clin Biochem* 2013

Hb Bart's ranges and associated α-globin gene status depending on hemoglobin plateforms

	Tanphaichitr VS, et al. Birth Defect (1988)		Sriroongrueng W, et al. Southeast Asian J Trop Med Public Health (1997)		M.J. Rugless, et al. Hemoglobin (2006)		Charoenkwan P, et al. Blood Cells, Molecules, and Diseases (2010)		Present Study	
	N	% Hb Bart's	N	% Hb Bart's	N	% Hb Bart's	N	% Hb Bart's	N	% Hb Bart's
Total	406		285		103		562		350	
Normal α-globin gene	326	0-4.9	238	0	30	0.5-5.6	399	0-0.1	306	0-1
Single α-globin gene defect	63	0-3.7	31	0-5.30	46	1.5-5.7	120	0-1	22	0-1.5
Two α-globin genes defect	16	4.1-9.8	14	2.29-9.00	27	6.1-11.9	34	3.2-12.9	19	5.2-8.7
Deletional Hb H disease	Not available		2	21.05-21.07	Not available		9	23.4-29.4	3	23-33
Hb Bart's Hydrops fetalis	1	97.5	Not available		Not available		Not available		Not available	
Hb Isolation Technique	Starch gel EP		Starch gel EP		HPLC		IEF		IEF	
Hb Measurement Technique	Cellulose acetate		Cellulose acetate		HPLC		Isoscan®		Isoscan®	
Molecular technique	Southern blot hybridization		Southern blot hybridization		Southern blot hybridization, α-globin sequence analysis		Multiplex PCR		Multiplex PCR, PCR-RFLP	

Diagnosing heterozygous β- thalassemia in the newborns

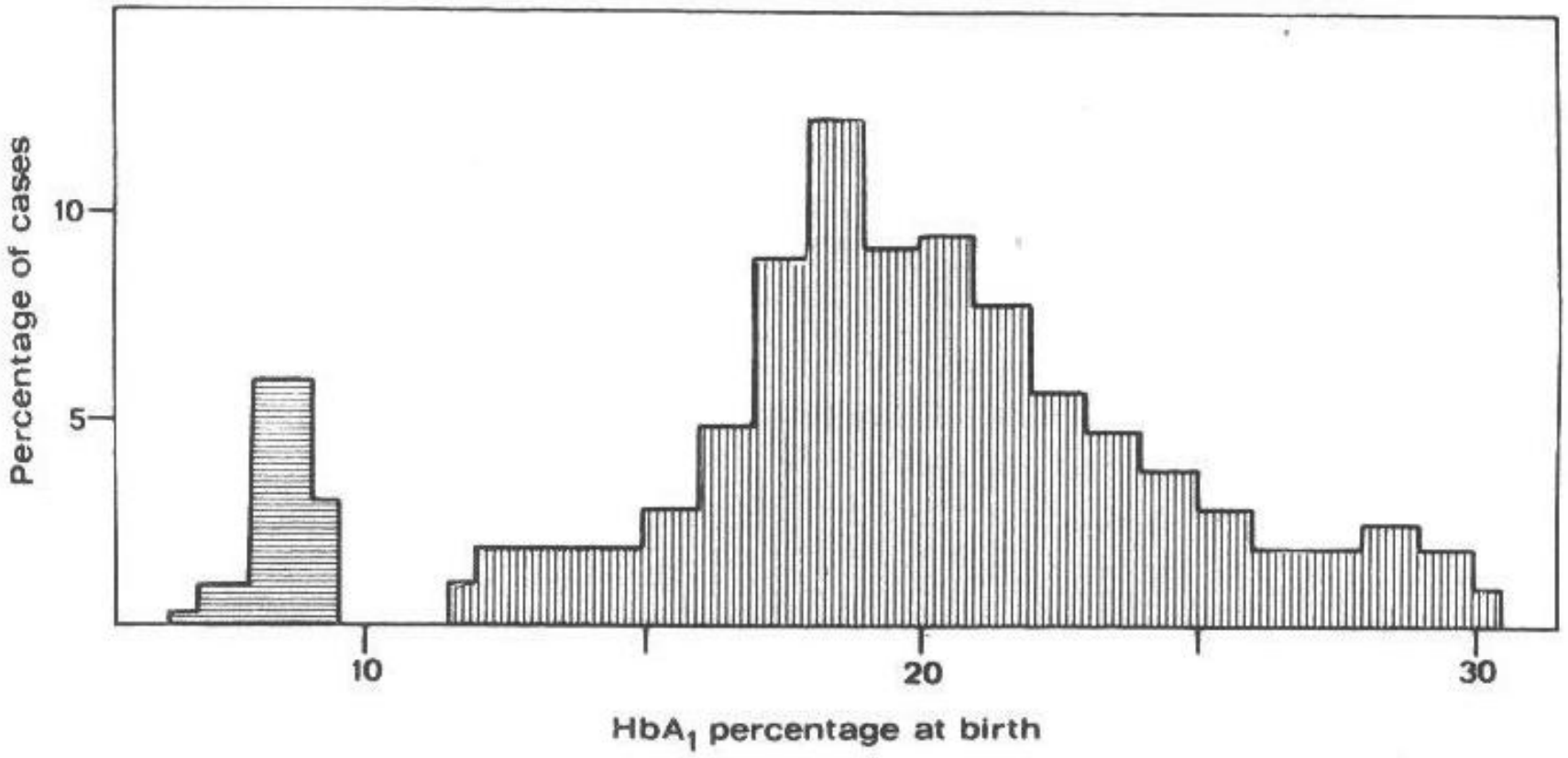
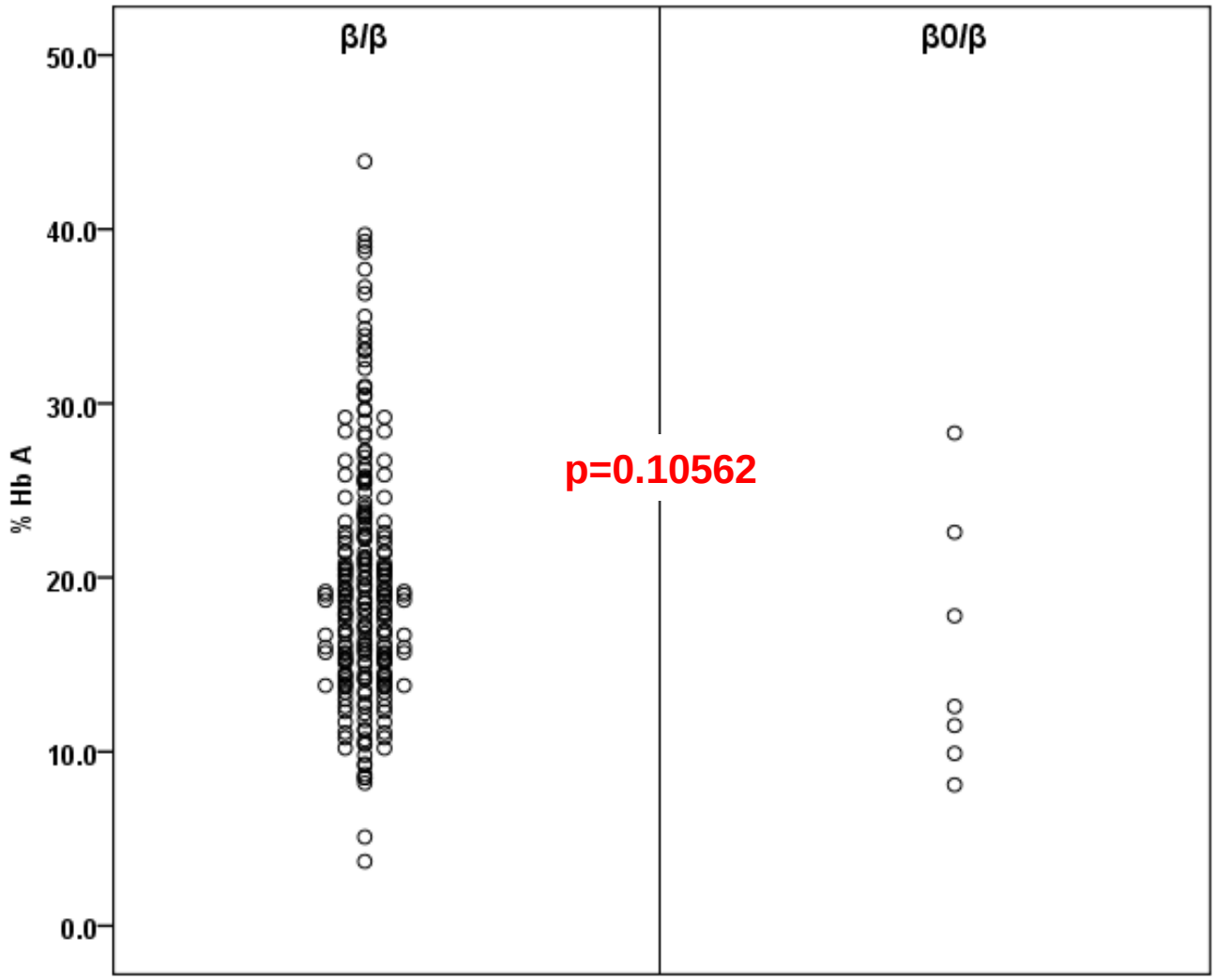


Fig. 4. Distribution of HbA_1 values at birth (734 entries). The peak centered around 9% (horizontal stripes) corresponds to β-thalassamic heterozygous, the one at 20% (vertical stripes) to normal newborns.

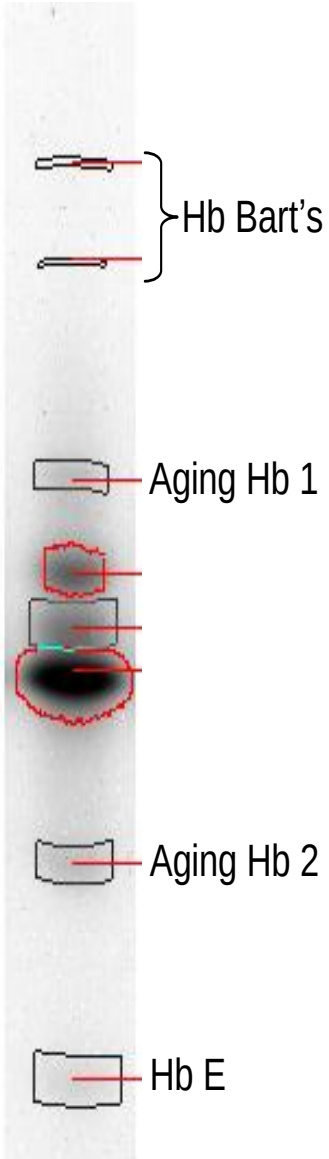
Gianfranco C., et al. J Hematol. (1982)

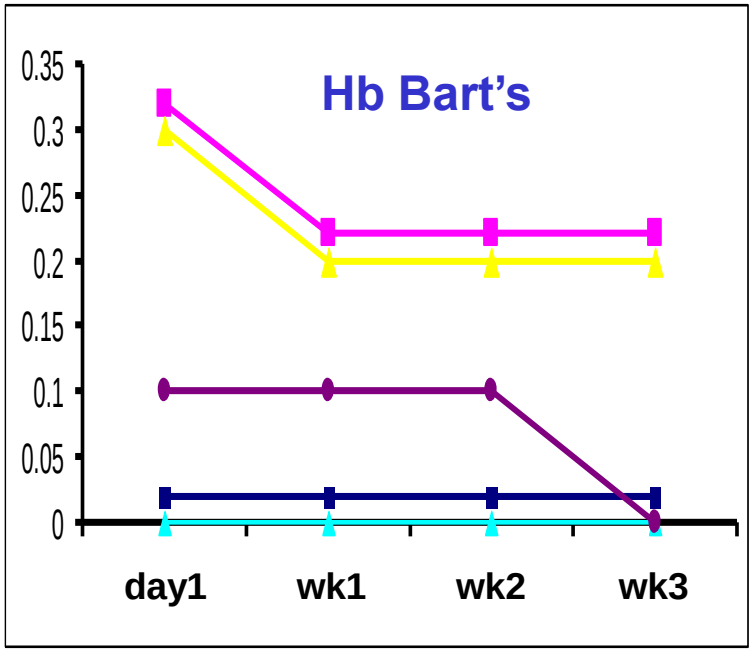
Diagnosing heterozygous β- thalassemia in the newborns

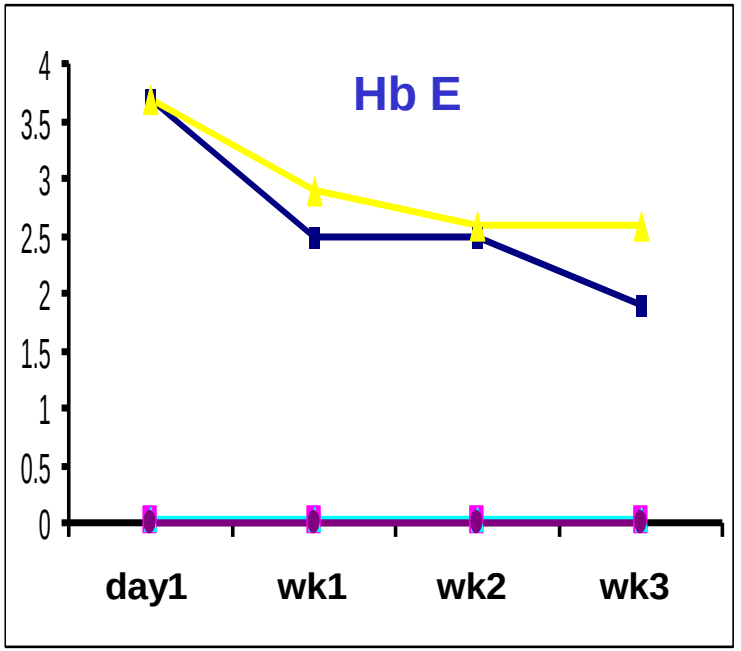


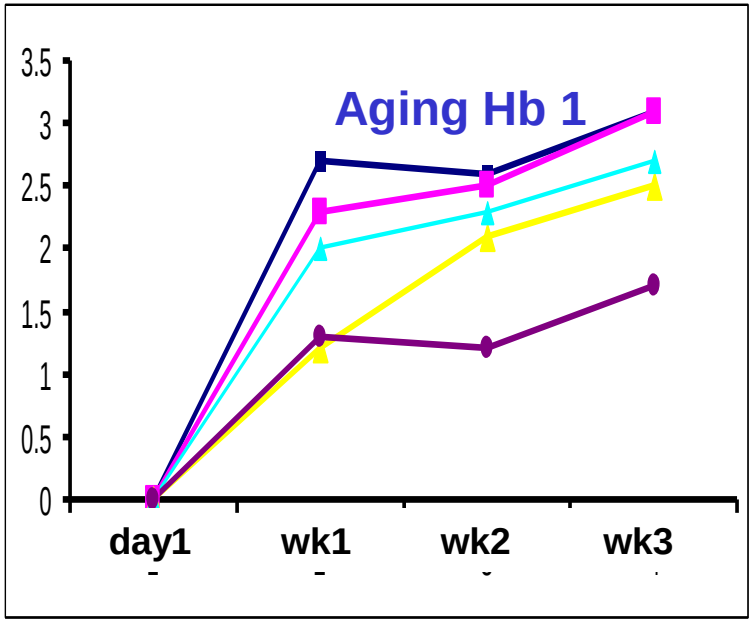
Comparison of Hb A level of normal newborn and the newborns with heterozygous β-thalassemia

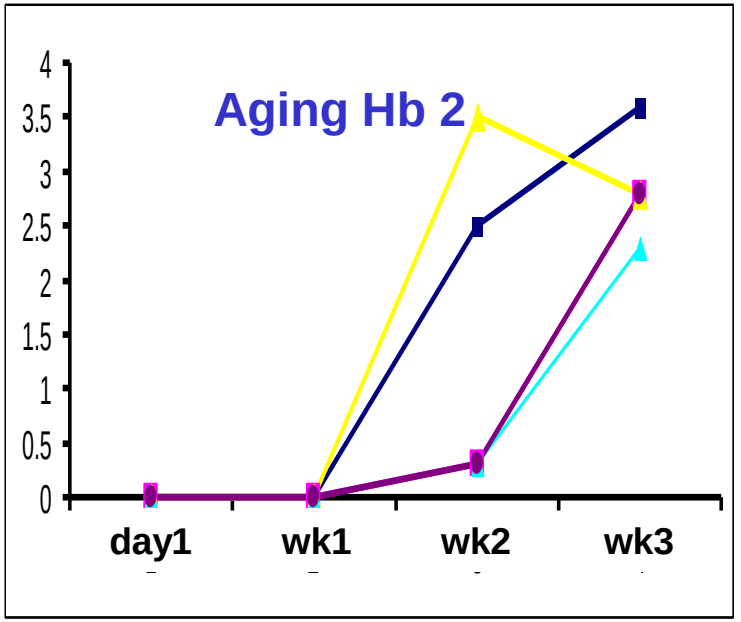
Stability of hemoglobin in dried blood spot studied by IEF











Neonatal screening with IEF can diagnose Hb E, EE & β^0 thal/Hb E $\pm$ α thalassemia

Hb E mutation	α-globin gene	Charoenkwan P, et al. (2010)			Present Study		
		N	%Hb Bart' s	% Hb E	N	%Hb Bart' s	% Hb E
β^E/β	**Normal**	41	0-0.3	0.9-2.9	72	0-1	2.0-4.5
	Single gene defect	19	0-0.13	0-0.5	5	0.3-1.0	1.5-1.7
	Two genes defect	2	4.2, 12.9	1.8, 0.5	5	5.9-6.9	1.5-1.7
	Three genes defect	2	23.8, 29.4	1.0, 2.1		NA	
β^E/β^E	**Normal**		NA		3	0-0.6	5.1-5.6
	Two genes defect				1	6.8	4.8
β^0/β^E	**Normal**	1	-	2.2		NA	

Punyanuch Pronpanich, MSc thesis, Mahidol U. 2010

Summary

- There is limiting efficacy of our current P&C program in antenatal period

- Carrier detection program can be performed at other groups: reproductive & newborn screening

- NB screening provides a potential effective model for future Thai generation as it can be incorporated into our existing NB screening program

- Further study is underway to provide a comprehensive management scheme for NB-4-Thal Health Service

Acknowledgements

Vip's Laboratory

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Suchada Ruengleung
Nuntawut Chat-Utai
Rapeepun Shompoopuong
Kalaya Tachavanich
Warapron Glomglao
Supachai Ekwattanakit

MRC Molecular Haematology Unit

Douglas R. Higgs
Chris Fisher
Helena Ayyub

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National Research University, Thailand

CDC: Center for Disease Control & Prevention, USA

- Routine testing of all newborns for some of the hemoglobinopathies is performed by the state-based newborn screening (NBS) programs since 2006
- Although screening for sickle cell disease (SCD), one of the hemoglobinopathies, has been included as part of NBS in all 50 states, screening for α- and β-thalassemia, is currently performed in only a few states.
- In addition, many people at risk for a hemoglobinopathy who live in the United States were born either before NBS began in their state, or in a country without NBS.
- For these reasons, the actual number of people in the United States with hemoglobinopathies, and the associated public health impact, are unknown.

CDC: Center for Disease Control & Prevention, USA

There is no ongoing monitoring system for hemoglobinopathies. This lack of a monitoring system makes it difficult to:

- Identify people with these conditions,
- Monitor use of healthcare services and any resulting changes in health or quality of life, and
- Understand the impact of these conditions on the healthcare system.