APPENDIX A

A MODIFIED MICROCOLUMN CHROMATOGRAPHY FOR HB A_2 DETERMINATION

DEAE-Sephadex A50 microcolumn chromatography is a standard technique routinely used, to determine the level of Hb A_2 [5] for thalassemia screening at Maharaj Nakorn Chiang Mai Hospital, Chiang Mai University. A modified microcolumn system is routinely used for thalassemia screening. Column and buffer reservoirs are made of a 3.5 ml and 10 ml disposable syringe, respectively, connected to each other as shown in **Figure A1**. A modified microcolumn packed with DEAE sephadex A50 that is well equilibrated with 0.05M Tris-HCl 0.01% KCN buffer pH 8.5 (buffer pH 8.5) prior to use.

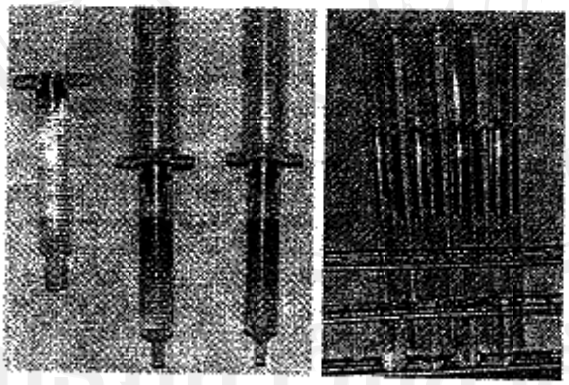

Figure A1 A Modified microcolumn

The hemolysate is prepared by dissolving 30 µl of packed cell in 2 ml of Tris-HCl buffer pH 8.5. The absorbance of a hemolysate dilution titer 1:100 is measured at 415 nm by spectrophometer and is labeled as OD_1. Determination of the amount of HbA_2 was done as follows; 10 ml of Tris-HCl buffer pH 8.5 is added into the column reservoir, after 5 ml of this Tris-HCl buffer solution has passed through the column then 1 ml of the hemolysate is added. The Hb A_2 is eluted by Tris-HCl buffer solution pH 8.2. With the column's dimention used here, about 20 ml of Tris-HCl buffer solution pH 8.2 is needed for the completed elution of Hb A_2. Two of 10 ml fraction are collected and the absorbance of the later fraction was measured at 415 nm as OD_2. The ratio of Hb A_2 can be calculated using the following equation, which has been developed by T. Sanguansermsri et.al. 2000 [7]:

$$\% \text{ Hb } A_2 = 20 \times OD_2/OD_1$$

APPENDIX B

HB E SCREENING TEST

Hb E screening test is modified from the microcolumn DEAE Sephadex A50 chromatography method. It has been used for screening of Hb E cases by applying a 40 μl blood sample dissolved in 5 ml of Tris-HCl buffer pH 8.5 into a Pasteur pipette containing DEAE Sephadex A50 of 5 cm high when the blood sample moves through the column, Hb is adsorbed on the sephadex resin. A 5 ml of Tris-HCl pH 8.5 is applied in order to the Hb. Then Hb E is eluted with 10 ml of Tris-HCl buffer solution pH 8.2. The red color band shown indicates type of Hb in the order of Hb A, Hb F, Hb H and Hb Bart. The colorless of DEAE Sephadex A50 at the lower part of the column indicates no Hb E in the blood sample as shown in **Figure B1**.

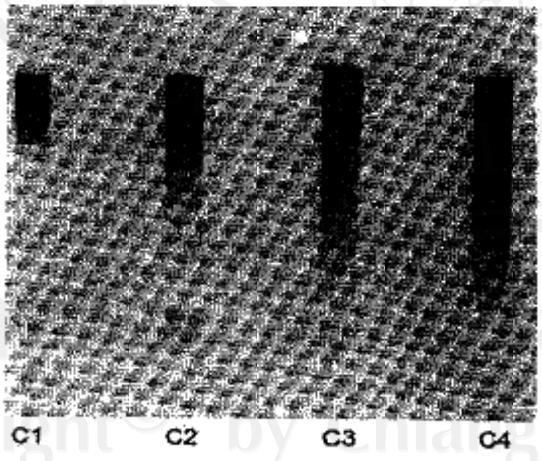

Figure B1 Hb E screening test; C1 is Normal blood (AA), C2 is Hb E trait (AE), C3 is Hb E/beta thalassemia (EF) and C4 is Hb E homozygous (EE).

APPENDIX C

ONE-TUBE OSMOTIC FRAGILITY TEST

The principle of one-tube Osmotic fragility test (OFT) is based on the limit of hypotonicity, which the red cell can withstand. It is a rapid, simple and cost effective screening test can be used for thalassemia screening, as described in the following procedure.

Procedure

1. Prepare buffer saline solution

- Stock solution : 10% NaCl buffer solution was prepared by dissolving 90.00 g NaCl, 13.65 g Na_2HPO_4 and 0.43 g $NaH_2PO_4.2H_2O$ in 1000 ml distilled water
- 1 % Buffer saline solution: 50 ml of Buffer saline stock solution was diluted to 1% by adding distilled water to the final volume of 500 ml.
- Working buffer saline solution (0.36% NaCl): It was prepared by adding distilled water into 180 ml of 1% Buffer saline solution to the final volume of 500 ml.

2. Prepare about 0.5-1 ml of the packed red cell in EDTA

   This could be achieved by centrifugation of red cells at 3000 rpm for 5 minute. After centrifugation, note the extent of colouration of the supernatant and the presence/absence of packed red cell.

3. Use pasteur pipette aspirate 10μl of packed red cell into a working buffer saline buffer tube. Allow the tube to stand for 15 second in front of the newsprint and visually observe the result.

4. Interprete the result as following;

Negtive: If the letters are visible through the solution (86-100% hemolysis) as shown in **Figure C1 (a).**

Positive: If the letters are not visible through the solution (< 85% hemolysis) as shown in **Figure C1 (b)**

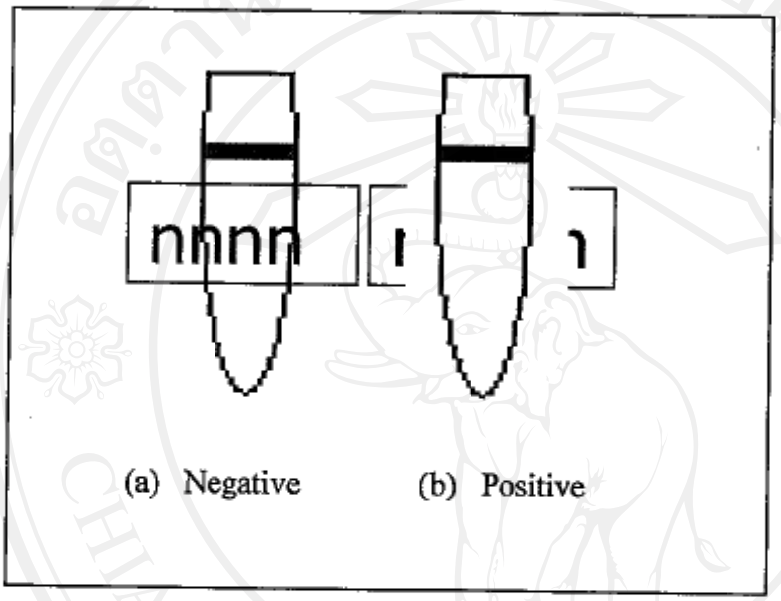

Figure C1 OF test results (a) Negative OF test result
(b) Positive OF test result

APPENDIX D

MATERIAL SAFTY DATA SHEET: POTASSIUM CYANIDE

KCN is added into buffer solution to preserve blood and to change iron in heme into Fe^{3+} that can be detected by a spectrophotometer. It is a toxic chemical and its properties are described as follows;

Description:

Synonyms: Potassium cyanide, solid; hydrocyanic acid, potassium salt

CAS No.: 151-50-8

Molecular Weight: 65.12

Chemical Formula: KCN

Hazard Class: (6.1) poisonous materials, (8.0) corrosive

Physical data

Appearance: White, amorphous, lumps, granular powder, or crystals granular powder or crystals

Melting point: 634 C

Vapour density: 2.2

Specific gravity: 1.52

Uses and Occurrences:

Extraction of gold and silver from ores

Reagent in analytical chemistry

Insecticide and fumigant

Electroplating

Reactivity and Fire Risks:

- Not combustible, but upon decomposition or contact with acids, releases highly flammable and toxic hydrogen cyanide gas
- Not considered an explosion hazard, but if heated with chlorates or nitrates may cause an explosion
- Do not use carbon dioxide to extinguish. Carbon dioxide can react with material and produce hydrogen cyanide.
- Use alkali dry chemical to extinguish fires involving this chemical

Health Hazards:

- OSHA PEL: 5mg/m^3
- IDLH: 25mg/m^3
- Highly toxic, may be fatal if inhaled, swallowed or absorbed through skin
- Cyanide poisoning can cause a deceptively healthy pink to red skin color. Cyanosis (blue discoloration of the skin) is associated with severe cyanide poisoning.
- Corrosive to respiratory tract, gastro-intestinal tract, skin and eyes

- Inhalation may cause blood, central nervous system and thyroid changes.

Personal Protective Equipment:

- Wear impervious protective clothing, including boots, gloves, lab coat, apron or coveralls to prevent skin contact
- Use chemical safety goggles and/or full face shield where dusting or splashing of solution is possible
- If exposure limit is exceeded wear a supplied air, full-facepiece respirator, airlined hood, or full-facepiece self-contained breathing apparatus

Inspection and Storage Tips:

- Keep in a tightly closed container in a cool, dry, ventilated area
- Do not store under sprinkler systems, water could lead to cyanide solution runoff and can produce dangerous amounts of hydrogen cyanide
- Store away from incompatibles including: acids, iodine, peroxides, permanganates, alkaloids, chloral hydrate and metallic salts

APPENDIX E

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

The thalassemias are hereditary conditions due to the mutations of the lost of the production of the α-globin or β-globin chain of hemoglobin. Thailand has high frequencies of thalassemia. β-thalassemia and Hb Constant Spring are distributed through out the region at low percentages (1-8%), but α-thalassemia and Hb E are occurred more often. α-thalassemia is mostly found in northeastern part of Thailand at a frequency of 30-40%. The North mostly found Hb E at a frequency of 13%. The thalassemia is an important clinical problem. The symptoms are breathlessness, fatigue and enlargement of liver and spleen. In some cases, the patients are cured by blood transfusion or operation of spleen and other treatments but that usually need to be done many times. The syndromes and treatment of thalassemia can physically, emotionally and economically affect the patient and their families. Therefore, thalassemia is one of the most concerns in the medical and clinical system of the country. The thalassemia screening program is necessary to control and prevent thalassemia from spreading further. Screening tests are the priliminary tests that are cheap and relatively quick. The objective of screening test is to differentiate normal persons from patients so that unnecessary detail analysis can be avoided. In the proposed system, flow injectin analysis (FIA) coupled with a reduced volume chromatographic column for separation of hemoglobin has been developed. This

technique was used to screen for some types of thalassemia that usually found in Thailand such as beta thalassemia trait, Hb E trait and Hb E homozygous. It was demonstrated that this technique could be an alternative economic method for thalassemia screening with the benefits of a simple, low cost and fast system. Therefore, thalassemia screening system is potentially useful for developing regions such as Thailand and neighboring countries.

APPENDIX F

A SIMPLE FLOW INJECTION - REDUCED VOLUME COLUMN SYSTEM FOR HEMOGLOBIN TYPING

Boonraksa Srisawang, Prachya Kongtawelert, Supaporn Kradtap Hartwell, Jaroon Jakmunee and Kate Grudpan, *Talanta* **60** (2003) 1163-1170.

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A simple flow injection-reduced volume column system for hemoglobin typing

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Abstract

A flow injection (FI)-reduced volume column system was developed for hemoglobin (Hb) typing to be used as an initial screening method for thalassemia. The column was packed with 140 μl diethylaminoethyl (DEAE)-Sephadex A-50 ion exchange beads. Hb can be separated using Tris–HCl buffer solution with pH gradient 8.5–6.5 and then monitored spectrophotometrically at 415 nm. The hemolysate of 40 blood samples from packed red cells were screened for thalassemia by determining the amount of HbA_2 and HbE present. The proposed system was able to predict positive test results from those samples with β, E-trait and EE homozygous thalassemia, Hb types that were independently identified following the conventional method at the hospital laboratory. Advantages of the proposed system over the conventional column technique include low amount of reagents and blood sample needed, short analysis time and low cost. Each analysis required only 80 μl of 50 times diluted packed cells, which is equivalent to 1.6 μl undiluted packed cells, and it can be completed in only 35 min. This simple FI-reduced volume column system was demonstrated to be an economic alternative system for Hb typing to initially screen some types of thalassemia such as β-trait, E-trait and EE-homozygous which are commonly found in Thailand.

Keywords: Flow injection; Hemoglobin typing; Reduced volume column

1. Introduction

Mutations of protein structure of the hemoglobin (Hb) are inherited through ancestor genes and cause many blood-related disorders such as Sickle Cell Anemia, and thalassemia. In Southeast Asia, Africa and Middle East, thalassemia trait is commonly found [1,2]. People with thalassemia trait are generally without health problems. However, if both parents have Hb variants (i.e. thalassemia trait), there is 25% chance that the baby will have homozygous variant (i.e. thalassemia disorder) [3].

Techniques commonly employed in the hospital to indicate the existence of thalassemia in patients are cellulose electrophoresis, micro-column chromatography and HPLC [4,5]. Electrophoresis can

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qualitate but cannot conveniently quantitate for different types of Hb. It is used to find out the exact type of thalassemia after the indication of having thalassemia was found. On the other hand, HPLC technique can be used to qualitatively and quantitatively analyze Hb but it requires an expensive instrumentation. Separation of Hb using DEAE-Sephadex column is well established and it is normally done to screen for thalassemia before performing further examinations. However, the conventional column technique involves analyzing many fractions of eluate collected batch-wise leading to long time, high amount of reagents and sample consumption. This work attempts to develop a simple, low cost and fast system to perform Hb typing.

In the proposed system, flow injection (FI) analysis coupled with a reduced volume chromatographic column for Hb separation has been developed. A flow based-system dramatically decreases the analysis time and can be automated [6,7]. Its closed system also reduces the possibility of sample contamination [8,9]. Hb typing is achieved using a reduced volume micro-DEAE-Sephadex ion exchange column that requires very small volume of diluted blood, which in turn generates only small amount of biological hazardous waste. The amount of Hb can be spectrophotometrically monitored at 415 nm. The technique is used to screen for some types of thalassemia based on abnormally high ratio of HbA_2 and HbE relatively compared with total amount of Hb as percentage. Comparison of the results with those obtained from the larger conventional column technique, indicates the possibility of applying the proposed system to initially screen for some types of thalassemia such as β-trait, E-trait and EE-homozygous before further conducting more expensive and conclusive testing.

2. Experimental

2.1. Combination of methods for thalassemia diagnosis

In regions where routine thalassemia testing is most needed due to its prevalence in the populations, such as in Southeast Asia and Africa, it is often also the case that economic restrictions on the medical systems prevent the use of the latest technologies that exist. In the laboratories that are not well equipped with more technically complicated instrumentation such as HPLC, diagnosis of Hb type is normally accomplished by conducting several different tests in combination, namely Osmotic Fragility Test (OFT), DEAE-column separation, HbE screening test (E-screen) and Polymerase Chain Reaction (PCR). OFT is a preliminary test based on the slower rupture rate of red blood cells of patients with thalassemia (positive) in hypotonic salt solution as compared with normal red blood cells (negative). DEAE-column separation can estimate percentage of HbA_2+HbE (positive when higher than 3.5%). E-screen is similar to the DEAE-column technique but the working buffer has precise pH that is more specific for HbE separation. Normal blood has less than 10% HbE (negative). Blood samples that have 25–30% HbE are considered as having HbE trait (positive) while those that have 70–90% HbE are identified as EE homozygous (positive). PCR-fluorescence spectrophotometry is used to indicate α-thalassemia gene in the patients who have normal level of HbA_2 with negative E-screen test. The relationship between results of each test and the diagnosis of Hb type is summarized as shown in Table 1.

2.2. Materials and apparatus

All pump tubings were tygon tubings (Saint-Gobain Performance Plastics, USA). The rest of the flow lines were assembled from PTFE tubings (Cole Parmer, USA). A peristaltic pump (FIA-lab, USA) was used to deliver buffer solutions. A reduced volume micro-column was made of acrylic piece into the dimension of 3 mm i.d. and 2 cm long. This dimension is much smaller than conventional column, which is about 1 cm i.d. and 5 cm long. It was packed with 140 μl of 40–120 μm DEAE-Sephadex A-50 beads (Phamasia Biotech, Sweden) which was about 20 times less than the amount of beads needed for packing a larger conventional column. Both sides of the column were sealed with cotton wool. Samples were

Table 1
Summarization of test results with diagnosis of Hb type

OFT	HbA_2 (conventional micro column)	E-screen	PCR	Hb type
+	–	–	–	A_2 normal
+	+ (>3.5%)	–	–	β-trait
+	+ (>60%)	+ (70–90%)	–	EE homozygous
+	+ (>3.5%)	+ (25–30%)	–	E-trait
+	–	–	+	α

introduced by means of a six port injection valve with an 80 μl sample loop. The flow through cell (HELLMA, Germany) with 1 cm path length was placed in the Spectronic 21 (Spectronic Instrument, USA). The detection of Hb was done at 415 nm. The absorbance data were converted to voltage and recorded with a computer software (Metex Corp., USA) installed in a personal computer (Compaq Presario 425). These data were transferred and integrated with Microsoft Excel (Microsoft Corp., USA).

2.3. Reagents

Tris–HCl 0.05 M buffer solutions of different pH values were prepared by dissolving 6.057 g Tris (hydroxymethyl) aminomethane (UBS, USA) and 0.1 g KCN (Riedel-De Haen Ag Seelze-Hannover, Germany) in 1000 ml distilled water and adjusted to the desired pH of 8.5, 7.5 and 6.5 with HCl (Merck, Germany). These three different pH buffers were used to create the pH gradient between 8.5 and 6.5.

2.4. Samples

Total of 40 blood samples (packed cells) were obtained from the Thalassemia Research Laboratories, Maharaj Nakorn Chiang Mai Hospital, Chiang Mai University where all subjects were routinely checked up. Each blood sample was hemolysed and diluted 50 times with Tris buffer pH 8.5 prior to use. The result from each sample was compared with the diagnosis result by the routine procedures of the hospital laboratory. The analytical procedures were independently run.

2.5. Manifold and operation steps

The diagram of a simple manifold used is shown in Fig. 1. Buffer solution pH 8.5 was pumped through the unloaded column to condition the column at a flow rate of 0.8 ml min^{-1}. Blood sample (80 μl) was injected into the system through a six port injection valve (V2). After the first peak of co-eluted HbA_2 and HbE appeared, the pH 8.5 buffer solution was replaced with pH 7.5 buffer solution by switching the valve V1. In between the gradient of pH 8.5 and 7.5, the HbA was eluted. Via the valve V1 then the buffer solution was again changed to pH 6.5 to elute out HbF. As a precaution the valve V3 can be used to drive off any observed air bubbles before they can get into the column.

3. Results and discussion

3.1. Elution profiles of hemoglobin

DEAE-Sephadex bead has diethylaminoethyl ($-OCH_2CH_2N^+H(CH_2CH_3)_2$) functional group that interacts with anionic groups on Hb and thus can retain all types of Hb. Different types of Hb contain different amount of net negative charge and, therefore, can be separated with the pH gradient elution. Upon elution using the more acidic buffer, containing more HCl, Hb becomes less negative and thus anionic groups of Hb captured by DEAE-bead can be exchanged with Cl^-. The order of Hb eluted from a DEAE-Sephadex column is HbA_2 co-eluted with HbE, then HbA and finally HbF [10,11].

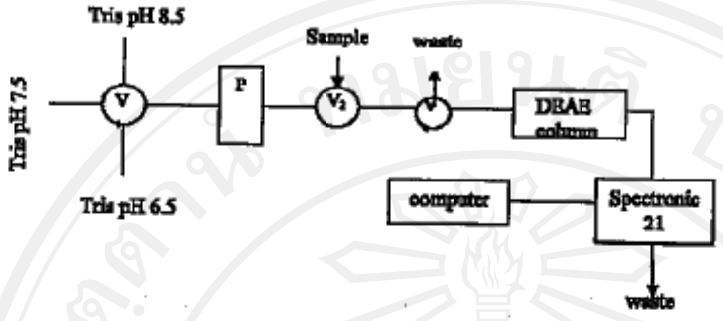

Fig. 1. Diagram of the simple FI manifold for Hb typing. V1, 4-ways valve; V2, six port injection valve; V3, 3-ways valve; P, peristaltic pump.

Examples of elution profiles of normal blood sample and that of EE-homozygous thalassemia patient obtained from the proposed system are illustrated in Fig. 2a and b, respectively. It should be noted that the *Y*-axis is in voltage that relates to transmittance and, therefore, peak height decreases with increasing of absorbance. The patterns of both elution profiles are similar but different in ratio of peak areas. Thalassemia patients have HbE that is coeluted with HbA_2 and, therefore, an abnormal blood sample shows larger first peak as compared with a normal blood sample.

3.2. Ratio of HbA_2 and HbE

Composition of Hb types in blood can vary depending on patient's age. Normal adult blood has been known to contain approximately 95–98% HbA, 2–3% HbA_2, and 0.8–2% HbF [12]. An individual with β-thalassemia trait usually has an elevated level of HbA_2, HbE and sometimes HbF along with the evidence of microcytosis and distinctive abnormal facial feature [2,4]. Patients with α-thalassemia, on the other hand, usually have HbA_2 and the same level as normal people have. Therefore, ratio of HbA_2 and HbE to total Hb cannot pinpoint what type of thalassemia the patient has without further examination.

From the elution profiles of blood samples, areas under each peak were integrated and the sum of those areas was counted as total Hb. The percentage of HbA_2+HbE was calculated from the ratio of area under HbA_2+HbE peak to the total area. Examples of calculation are also summarized in Fig. 2a and b. Even though ratio of HbA_2+HbE to total Hb cannot predict exactly what type of thalassemia the patient has, it can help indicate the existence of some types of thalassemia (i.e. β, EE homozygous, E trait types). The aim here is to utilize the benefits of the flow

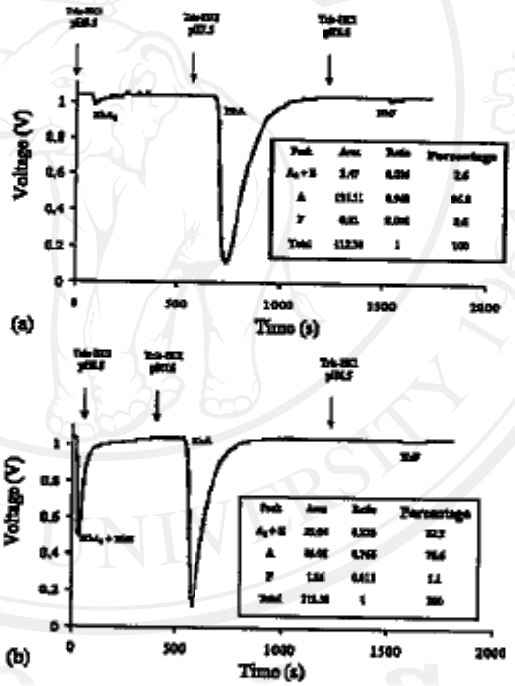

Fig. 2. FI-elution profiles of Hb in blood samples from (a) a normal adult and (b) an EE-homozygous thalassemia patient.

system combining with a DEAE-column technique and to show that the proposed FI-reduced volume column system can be used to initially screen for patients with some types of Thalassemia.

3.3. Evaluation of the proposed FI-system

In this work 40 adult blood samples (packed red cells) with positive OFT were examined without the prior knowledge of the type of Hb of each sample. Related to the hospital diagnostic results which were independently run, the correlation plot between the two systems, $R = 0.9271$, was obtained as shown in Fig. 3. The calculated percentages of HbA_2+HbE obtained from the proposed FI-reduced volume column system along with the results from a conventional column, E-screen and PCR are shown in Table 2(a) with a summary in Table 2(b). Two samples (one β-trait (18.4%) and one α-trait (17%)) were statistically eliminated from Table 2 because they gave results much higher than their standard ranges (β-trait 4–8%, α-thal-1-trait 2.5–3.5%) that are normally obtained using the HPLC. Because of the differences in the system characteristics and column size, the averages of HbA_2+HbE amounts obtained from different cases of thalassemia using the proposed system are lower than those obtained when using the conventional system, especially for the cases of E-trait and EE homozygous. The cause of deviation is currently under further investigation. However, the proposed system can differentiate normal and α-thal-trait samples (HbA_2+HbE < 3.5%) from abnormal samples (> 3.5%) and was still able to yield accurate positive test results. The level of HbA_2+HbE in EE homozygous samples was higher than in E-trait and β-trait samples, respectively, the same trends as indicated in the conventional column results.

For other types of Hb such as HbS, no testing could be conducted as there were no samples available in Thailand. However, according to a

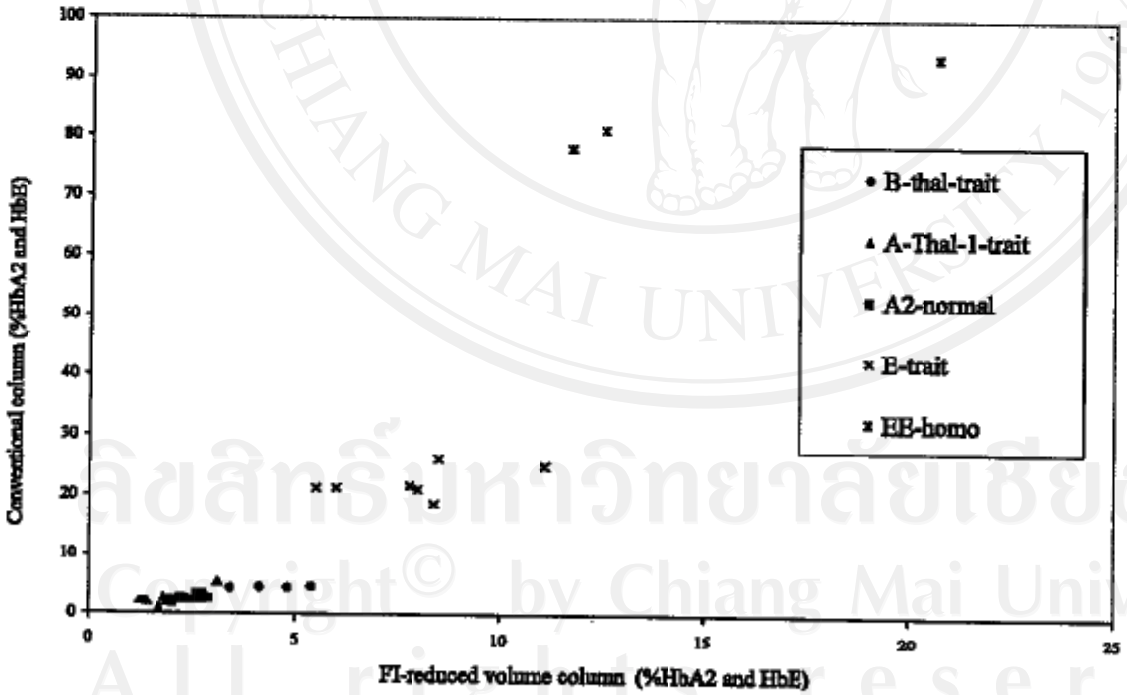

Fig. 3. Correlation plot between percentages of HbA_2+HbE obtained from the proposed FI-reduced volume column system and those obtained from the conventional column system.

Table 2
Comparison of calculated percentages of HbA_2+HbE peak areas from the proposed FI-reduced volume column system* and from the conventional column system*

Sample	Percentages HbA_2+HbE			E-screen*	PCR*	Hb type
	FI-red. V column*	Conventional column*	Ratio, FI-red. V: convention			
(a)						
A4	2.6	2.9	1:1.1	–	–	A_2-Normal
B6	1.3	2.2	1:1.7	–	–	A_2-Normal
B11	2.2	2.9	1:1.3	–	–	A_2-Normal
B13	1.9	2.5	1:1.3	–	–	A_2-Normal
B15	2.6	3.4	1:1.3	–	–	A_2-Normal
B16	2.7	3.4	1:1.3	–	–	A_2-Normal
C3	1.9	2.5	1:1.3	–	–	A_2-Normal
C25	2.8	2.8	1:1.0	–	–	A_2-Normal
D17	2	2.3	1:1.2	–	–	A_2-Normal
E6	2.6	2.4	1:0.9	–	–	A_2-Normal
E25	2.3	2.6	1:1.1	–	–	A_2-Normal
F18	2.4	2.5	1:1.0	–	–	A_2-Normal
C8	2.9	2.7	1:0.9	–	–	A_2-Normal
C5	3.4	4.5	1:1.3	–	–	β-Thal-trait
D3	5.4	4.8	1:0.9	–	–	β-Thal-trait
D9	4.1	4.7	1:1.1	–	–	β-Thal-trait
D18	7.3	18.4	1:2.5	–	–	β-Thal-trait
E24	4.8	4.6	1:1.0	–	–	β-Thal-trait
A9	1.8	2.9	1:1.6	–	+	α-Thal-1-trait
A8	3.1	5.5	1:1.8	–	+	α-Thal-1-trait
A28	2	2.5	1:1.3	–	+	α-Thal-1-trait
B2	2.2	2.5	1:1.1	–	+	α-Thal-1-trait
C2	1.7	1.1	1:0.6	–	+	α-Thal-1-trait
C11	1.4	2	1:1.4	–	+	α-Thal-1-trait
C12	1.2	2.2	1:1.8	–	+	α-Thal-1-trait
C16	2.7	2.4	1:0.9	–	+	α-Thal-1-trait
D2	1.3	2.2	1:1.7	–	+	α-Thal-1-trait
E7	1.9	2.1	1:1.1	–	+	α-Thal-1-trait
E10	2	1.9	1:1.0	–	+	α-Thal-1-trait
E20	8.5	17	1:2.0	–	+	α-Thal-1-trait
A1	5.5	21.3	1:3.9	+	–	E-trait
A23	8	21.1	1:2.6	+	–	E-trait
B4	8.4	18.7	1:2.2	+	–	E-trait
C13	6	21.4	1:3.6	+	–	E-trait
D20	11.1	25	1:2.3	+	–	E-trait
E19	8.5	26.1	1:3.1	+	–	E-trait
C7	7.8	21.7	1:2.8	+	–	E-trait
A39	12.5	81.2	1:6.5	+	–	EE-homo
C24	11.7	78.2	1:6.7	+	–	EE-homo
A37	20.7	93.8	1:4.5	+	–	EE-homo

Table 2 (*Continued*)

Hb type	HbA_2+HbE percentage				
	FI-reduced volume column*		Conventional larger column*		Standard range (HPLC)*
	Range	Average ± S.D.	Range	Average	
(b)					
A_2-normal (13)[a]	1.3–2.9	2.3±0.1	2.2–3.4	2.7	2.5–3.5
α-thal-1-trait (11)[a]	1.2–3.1	1.8±0.1	1.1–2.9	2.5	2.5–3.5
β-thal trait (4)[a]	3.4–5.4	4.1±0.2	4.5–4.8	4.6	4.0–8.0
E-trait (7)[a]	5.5–11.1	8.0±0.1	18.7–26.1	22.2	> 10
EE-homo (3)[a]	11.7–20.7	14.3±0.9	78.2–93.8	84.5	> 60

(a) Results from each samples are shown along with the results from E-screen*, PCR-fluorescence spectrometry* and Hb Type diagnosis that were done by the Thalassemia Research Laboratories, Maharaj Nakorn Chiang Mai Hospital, Chiang Mai University (+ is positive result and – is negative result). (b) Summarization of ranges of HbA_2+HbE percentages and average values obtained when using the proposed and conventional column systems. *Independently run. Note: there were no S.D. data for the results obtained from the conventional column.

[a] Number of samples for each Hb type.

previous study by Dozy et al. [5], the DEAE column can separate Hb by descending pH gradient between 8.5 and 7.0. The order of Hb eluted from the column were HbA_2, HbS, HbA and finally HbF, respectively, in that study. Similarly, in this proposed study, HbA_2, HbA and HbF were eluted in the same order based on a similar pH range. Therefore, this proposed method should be able to screen for HbS if it is contained within the sample.

Reproducibility of the system was tested by running seven replicates of three samples (normal, β-trait and E-trait) each of which represented different levels of HbE. R.S.D. of the method was found to be 3.4% for normal sample and 4.6% for both β-trait and E-trait samples.

3.4. Advantages and disadvantages

The DEAE column technique has two main disadvantages. Neither the proposed nor the conventional column systems can point out patients with α-thalassemia due to non-differentiated level of HbA_2+HbE from normal people, in which case PCR result is needed. Also, both the proposed reduced volume column and the conventional column systems have another similar inconvenience in that they have to be repacked after each run due to constriction of the flow caused by coagulation of some matrices in blood. However, using a fresh column each time will eliminate the carry over effect from the previous run.

In the proposed FI-reduced volume column system, the improvement comes from the reduction in the amount of beads used and the time it takes for column packing, both of which are minimized as compared with those of the conventional column. The proposed system offers other advantages such as ease of operation, low cost, small amount of sample, and fast analysis time. The analysis time using the FI-reduced volume column is about 35 min per sample as compared with 4 h using a conventional column. Amount of sample for each run was as little as 80 μl of 50 times diluted sample, a major improvement as compared with the 2 ml of undiluted packed cell that is needed for a conventional column technique.

4. Conclusion

The FI-reduced volume column system for Hb typing was developed. The system was used as an initial screening for some types of thalassemia such

as β-thal-trait, E-trait and EE-homozygous which are commonly found in Thailand. It was demonstrated that the proposed system could differentiate normal blood samples from abnormal ones. Although the cause of deviation between results from the proposed system and those from the larger conventional column technique normally performed in the hospital needs further investigation, the proposed system was still able to predict positive test results. This preliminary study shows that the proposed system offers some advantages over the conventional column technique, including a simpler instrumentation with ease of operation, shorter analysis time and lower amounts of sample and reagents needed. These benefits will help reduce the overall analysis cost and should be useful as an economic alternative technique for routine thalassemia screening involving a large number of blood samples.

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