

CHAPTER IV

RESULTS

1. Method validation

1.1 Selectivity

A representative of 6-MMP blank erythrocyte lysate sample in Figure 14 was compared with 6-MMP erythrocyte lysate sample in Figure 15. It was clear that responses of interferences in erythrocyte lysate sample was outside the retention time of 6-MMP (13.929 min), indicating the selectivity of the method to 6-MMP.

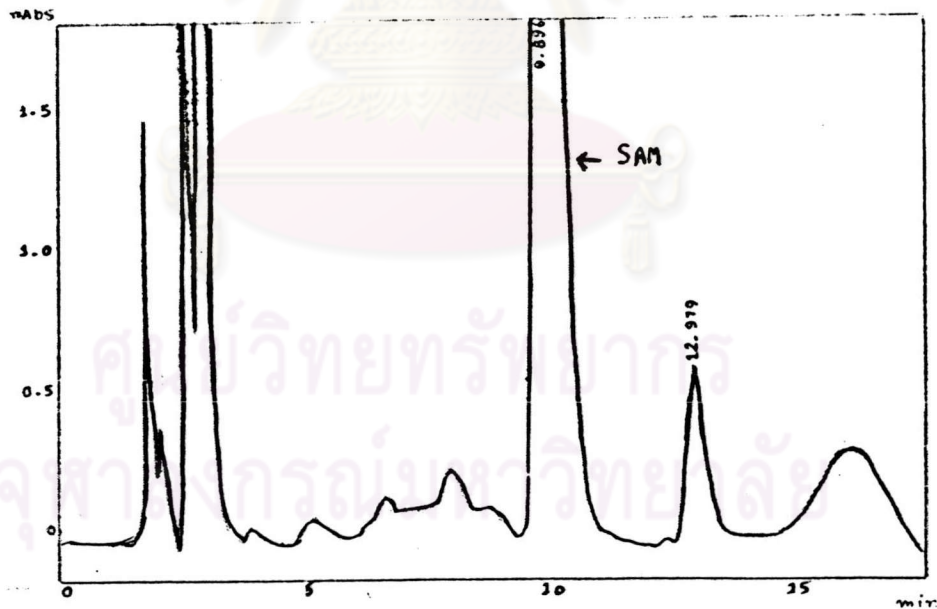


Figure.14 Representative chromatograms of standard condition incubation of red blood cell lysate in the presence of SAM (9.896) and without 6-MP.

1.2 Linearity

The calibration curve of the 6-MMP at range 50-1000 ng/ml was illustrated in Figure 17. This curve has correlation coefficient of 0.9999.

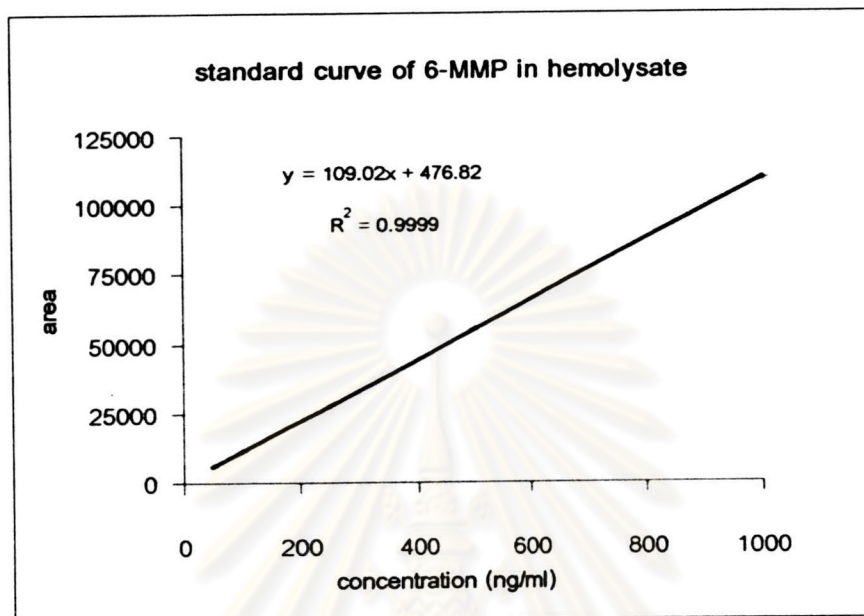


Figure.17 Standard curve of 6-MMP in erythrocyte lysate (conc. 50-1000 ng/ml)

1.3 Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD of 6-MMP was 10 ng/ml and LOQ was 25 ng/ml (Table.3).

Table.3 The value of %RSD and %Bias of LOQ

Conc. Of 6-MMP	%RSD	%Bias
25 ng/ml	6.42	15.06
50 ng/ml	4.26	3.23

1.4 Precision

Five replicate of 6-MMP erythrocyte lysate sample at 50, 100 and 500 ng/ml was detected by HPLC method, %RSD (precision) was presented in table 4

Table.4 The value of precision (%RSD)

Concentration of 6-MMP (ng/ml)	% RSD	
	Intra-assay	Inter-assay
50	0.14	6.34
100	0.12	2.55
500	0.14	7.87

1.5 Accuracy

Six replicate of 6-MMP erythrocyte lysate sample at 50, 100 and 500 ng/ml was measured by HPLC method, the result showed % Bias (accuracy) of this method (Table 5).

Table.5 The value of accuracy (%Bias)

Concentration of 6-MMP (ng/ml)	% Bias
50	5.35
100	7.23
500	9.27

1.6 Absolute Recovery

The result displayed % Absolute Recovery of this method (Table 6)

Table.6 The value of %Absolute Recovery

Concentration of 6-MMP (ng/ml)	% Absolute Recovery
50	99.82
100	99.47
500	94.89



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2. Thiopurine methyltransferase activity in erythrocyte of Acute Lymphoblastic Leukemia children

To illustrate result of thiopurine methyltransferase activity, a histogram and probit graph were shown (Fig18, 19). Probit analysis has shown that thiopurine methyltransferase activity in erythrocyte was divided into 2 groups by cut point approximately 15 unit/ml pRBC/hr. The first group has intermediate activity, 6.66%, (range ~ 5-15 unit/ml pRBC/hr), the second has high activity, 93.37%, (range > 15 unit/ml pRBC/hr).

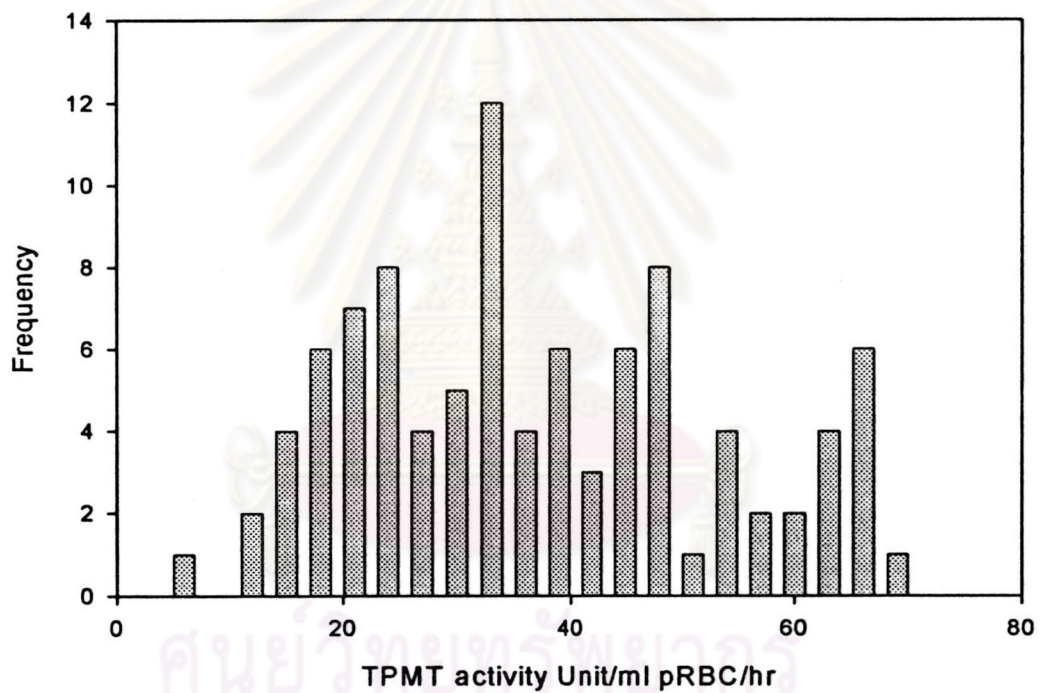


Figure.18 Frequency distribution histograms of erythrocyte TPMT activity of 90 ALL children

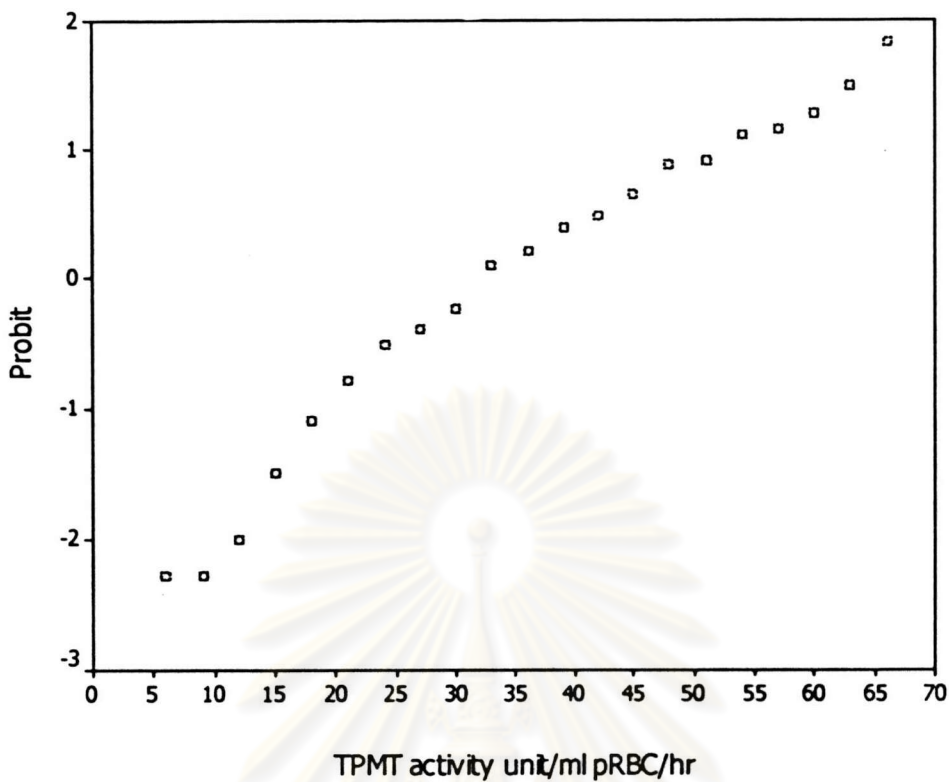


Figure.19 Probit plot of erythrocyte TPMT activity of 90 ALL children

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3. Evaluation of factor affecting Thiopurine methyltransferase activity

Gender

This study has shown the TPMT activity in 53 males and 37 females (Table 7). There was no significant difference of TPMT activity in male and female.

Table.7 Comparison of red blood cell TPMT activity between male and female

Gender	TPMT activity	
Male (n=53)	5.54-66.46 (36.58 ±14.81)	*NS
Female (n=37)	9.81-67.36 (31.34±16.43)	

*NS = Non-significant

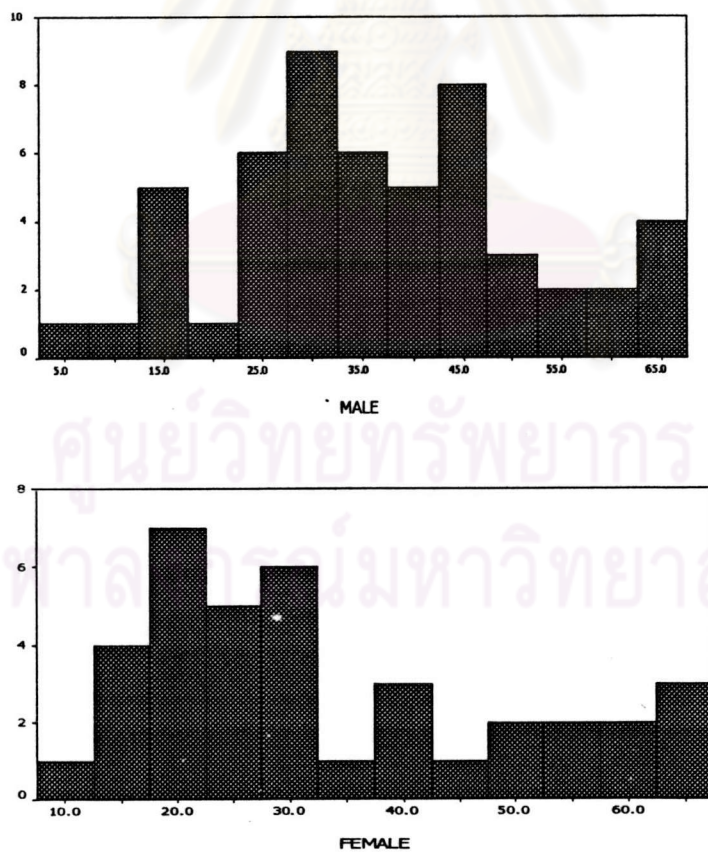


Figure.20 Histogram shown TPMT activity of male and female

Receiving 6-MP

This study displayed TPMT activity in 27 patients, not receiving 6-MP, and 63 patients, receiving 6-MP (Table.8). There was significant difference of TPMT activity in receiving and not receiving 6-MP.

Table.8 Comparison of red blood cell TPMT activity between receiving and not receiving 6-MP

	TPMT activity	p-value
off (n=27)	9.81-66.46(28.06 ±13.71)	0.013*
maintenance (n=63)	5.54-67.36 (38.24± 25.52)	

* = significant (p<0.05)

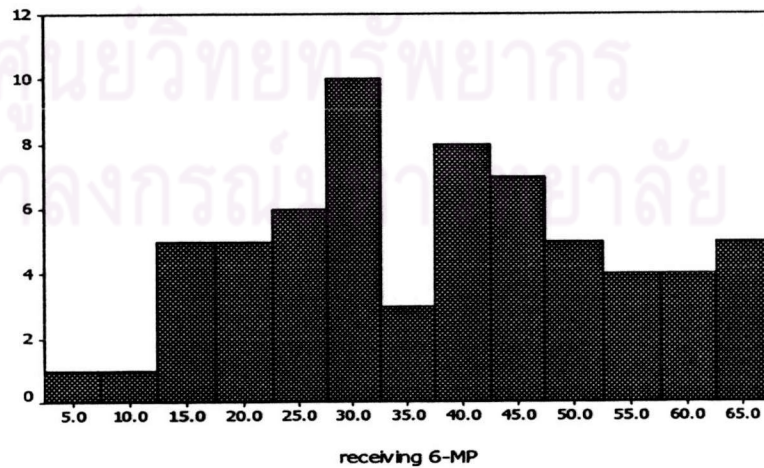
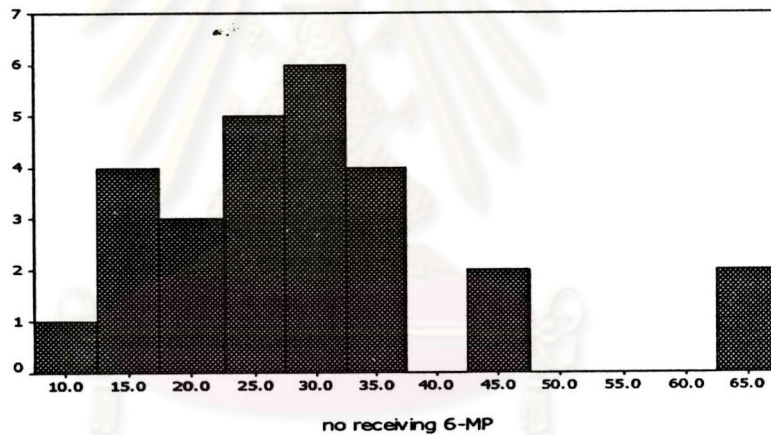


Figure.21 Histogram shown TPMT activity of receiving and not receiving 6-MP

4. The TPMT genotype of Acute Lymphoblastic Leukemia children

TPMT genotypes were determined for all individuals with PCR methods used to detect the mutant alleles (TPMT*2, TPMT*3A and TPMT*3C). The PCR product Table.9 summarized the mutant alleles found in 90 ALL children.

Table.9 Mutant alleles was detected in children with ALL

TPMT genotype	No. of patients	% patients
TPMT*1/TPMT*1 (Wild type)	84	93.33
TPMT*1/TPMT*2 ($G^{238} \rightarrow C$)	0	0
TPMT*1/TPMT*3A ($G^{460} \rightarrow C, A^{719} \rightarrow G$)	0	0
TPMT*1/TPMT*3C ($A^{719} \rightarrow G$)	2	2.22
Unknown	4	4.44

1 2 3 4 5 6 7 8 9 10 11

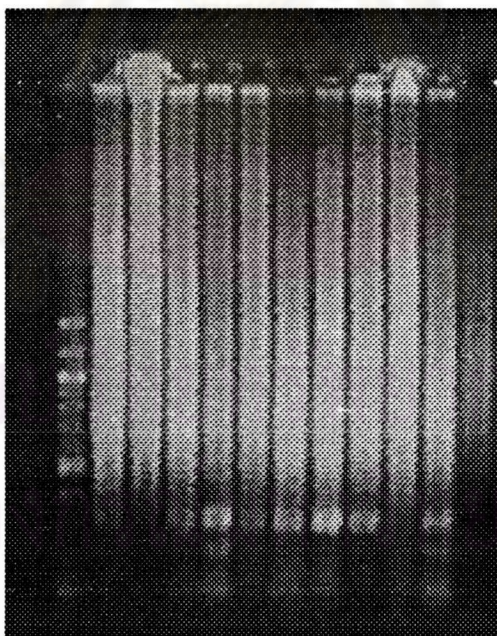


Figure.22 Polymerase chain reaction fragment length polymorphism (PCR-RFLP) of common thiopurine S-methyltransferase (TPMT) mutant alleles. Detection A719G, lane 1 marker, lane 5 and 11 were heterozygous mutation, other were normal case.

5. Correlation between genotype and phenotype of TPMT activity

In this study, most TPMT activity (phenotype) were correlated with genotype, except 4 samples had unknown genotype (Table.10).

Table.10 Relationship between TPMT genotype and activity (phenotype)

Enzyme activity (phenotype)	Genotype
High (>15unit/ml pRBC/hr)	TPMT*1 (wild type) n=84
Intermediate (5-15 unit/ml pRBC/hr)	TPMT*1/TPMT*3C n=2
	Unknown n=4

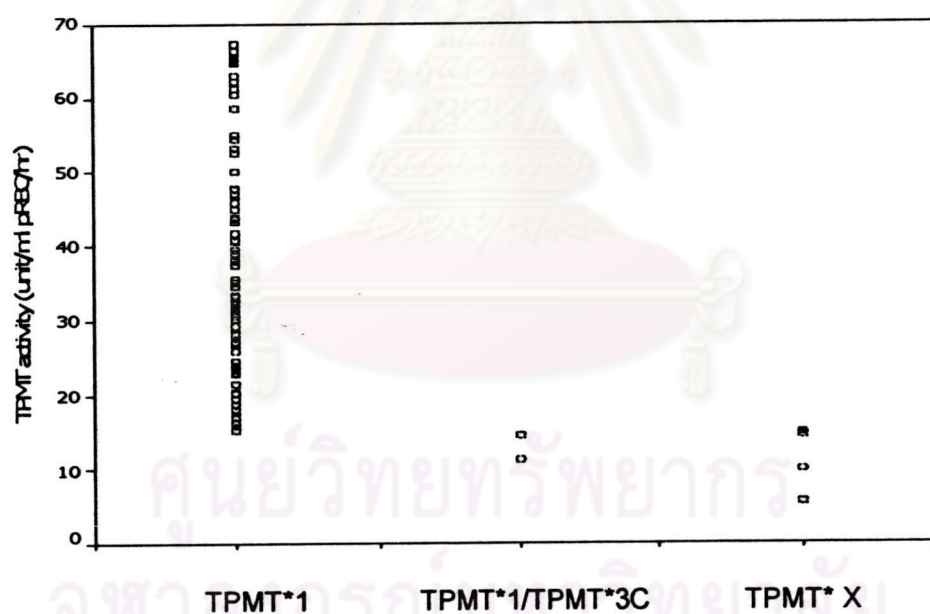


Figure.23 TPMT activity of each genotype