

## *The In-house TSH IRMA of Dried Blood Spots Part 3: Comparison Test*

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**S**ince TSH is an important parameter in the screening of Neonatal Congenital Hypothyroidism, the in-house IRMA for determination of TSH in dried blood spot specimen, which was developed to reduce cost, was reported in 1991<sup>(1)</sup>. During that time the Henning TSH-IRMAClon<sup>®</sup> Screening was one of the qualified commercial kit. The comparison between the Henning and the in-house techniques was then performed.

### **MATERIALS AND METHOD**

For the Henning TSH-IRMAClon<sup>®</sup> Screening Technique<sup>(2)</sup>, two spots (6 mm in diameter) of each sample (standards, quality controls, or unknown) were mixed with 750 µl elution buffer, and then agitated for at least 30 minutes. Each eluate was duplicatedly pipetted for 200 µl, transferred to its corresponding conical tube, and mixed with 50 µl anti-TSH solid phase and 50 µl labelled anti-TSH. The incubation was done for 2 hours at room temperature. It was then washed once with wash buffer. The radioactivity in each tube was measured for 1 minute. The unknown values were obtained by extrapolating from the standard curve.

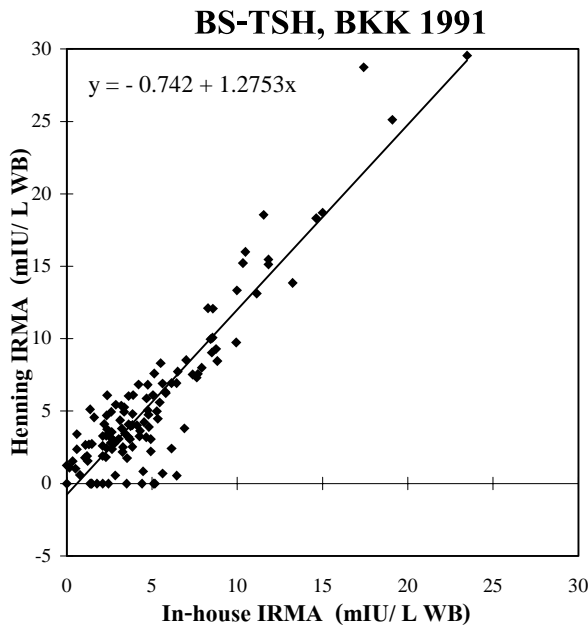
For the In-house technique, a duplicate of one spot (6 mm in diameter) of each sample was mixed with 100 µl assay buffer, 50 µl anti-TSH-I<sup>125</sup>, and 50 µl anti-TSH-solid phase. The incubation was done overnight at room temperature. The mixture was then washed twice with wash

buffer. The procedure afterwards was more-or-less the same as that of the Henning TSH-IRMAClon<sup>®</sup> Screening.

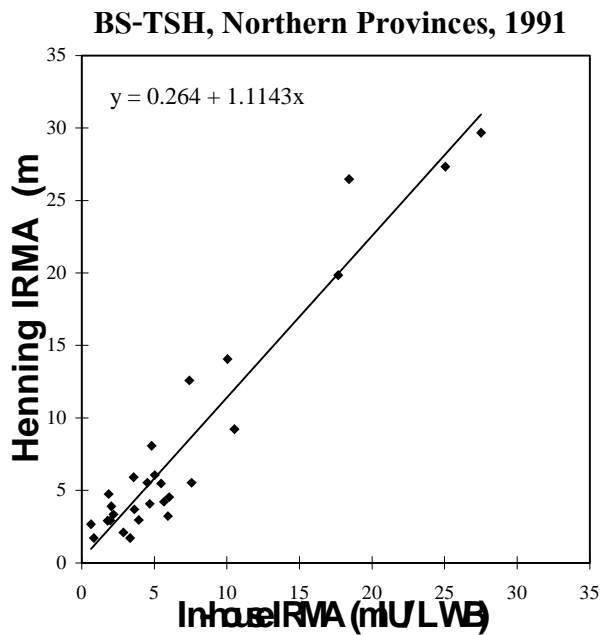
The neonatal blood spot samples were obtained from the Department of Gynecology, Siriraj Hospital, Bangkok (n=120) and from the northern provinces of Thailand (n=28). They were assayed by the two methods and the results were then compared. The statistical analysis was based on the Pearson Correlation<sup>(3)</sup> and the Intra-Class Correlation or Agreement Index<sup>(4)</sup> using the Microsoft Excel Version 7 software.

### **RESULTS**

The correlation coefficient of blood spot values of the Bangkok specimen obtained from the Henning and the in-house methods was 0.916 with the equation of Henning =  $-0.742 + 1.275 \text{ Inhouse}$ . The one for the provincial samples was 0.961, and Henning =  $0.264 + 1.114 \text{ Inhouse}$ . Those results were demonstrated in Figures 1 and 2, and Table 1.



**Fig. 1** The correlation of the blood spot TSH values of BKK specimens as obtained from the Henning TSH-IRMA<sup>®</sup> Screening and the In-house IRMA techniques.



**Fig. 2** The correlation of the blood spot TSH values of provincial specimens as obtained from the Henning TSH-IRMA<sup>®</sup> Screening and the In-house IRMA techniques.

The Intra-Class Correlation (ICC) or Agreement Index values of blood spot levels obtained from the two methods were 0.862 for the Bangkok specimen, and 0.943 for the provincial, as shown in Table 1.

**SUMMARY AND DISCUSSION**

To compare the developed method with a well-known and reliable commercial kit is one of the effective tools to evaluate the technique. The results showed that there was a highly satisfactory correlation between the Henning TSH-IRMA Clon® Screening which was one of the qualified commercial kits from Europe and the In-house techniques both for the Bangkok and provincial specimens. So were those of the ICC suggesting not only good correlation between the two methods but close values also.

Though only 28 provincial blood spot specimens were analyzed because of the limitation of the reagents of the Henning technique, both methods showed slightly higher

TSH levels than those of BKK at some extent. This may be because they came from the iodine deficient area. When the reference ranges<sup>(1)</sup> were assigned as follows: 0-15 mIU/L WB suggested normal (N), 15-25 transient or subclinical hypothyroidism (E), and > 25 hypothyroidism (H), 24 from 28 were interpreted as N, 1/28 E, and 3/28 H for the Henning method, whereas 24/28 N, 2/28 E, and 2/28 H for the In-house. The difference in the interpretation was 1/28 or 3.6 % and it was in the vicinity.

When the cost was concerned, the in-house technique had the obvious advantage over the Henning. While the first cost only 4 baht/ case, the latter could be around 70. This was because we had to order the kit(s) from abroad; then the air-freight, tax, etc were included in the price. Thus, the in-house technique is worth for the screening purpose either through its efficiency or expenses.

*Table 1 The blood spot TSH values determined by the two different methods*

Blood spots	n	Mean ± S.D. (mIU/L WB)		r	ICC
		Henning TSH-IRMA Clon® Screening	In-house IRMA		
Bangkok	120	5.64 ± 5.55	5.00 ± 4.00	0.916	0.862
Provinces	28	8.04 ± 8.08	6.97 ± 6.96	0.961	0.943

## REFERENCES

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