Evaluation of Neonatal Thyroxine and Neonatal Thyroid-Stimulating Hormone Radioimmunoassay Kits

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Hypothyroidism in both premature and full-term infants is known to surface within a short time after birth. If left untreated, this condition often leads to cretinism and, eventually, mental retardation. Our institution performs thyroid hormone screening on neonates in the hope that such problems may be avoided. Using blood-stained filter paper spots, various radioimmunoassay kits were evaluated to find one neonatal thyroxine and one neonatal thyroid-stimulation hormone kit which could yield reproducible results and low cost for the patient. The results obtained determined which kits are best suited to our needs in diagnosing hypothyroidism in infants.

Congenital hypothyroidism affects approximately 1 in 4000 infants, causing these neonates to become severly retarded if left untreated (*1–4*). As they grow older, hypothyroid children usually have IQs between 50 and 80, ataxic gaits, lumbar lordosis, myxedematous skin and tongues, and low body temperatures (*1,3,5–9*). These symptoms are probably due to an increase in brain cell density and a decrease in brain mass, resulting from abnormal and/or decreased nerve myelination (*6,10,11*). Recent federal legislation requires all children to be screened for hypothyroidism; in the state of Nebraska, the institution of birth is responsible for making sure that everyone has a neonatal thyroxine (NN T_4) test performed.

The endocrine glands that supply the thyroid hormones are sometimes referred to as the hypothalamic-pituitary-thyroid axis. The hypothalamus secretes thyrotropin-releasing hormone (TRH), which causes the anterior pituitary to secrete thyroid-stimulating hormone (TSH). The TSH, in turn, causes the thyroid to secrete tri-iodothyronine (T_3) and thyroxine (T_4). A normal feedback mechanism that detects high levels of T_3 and T_4 sends a signal to the hypothalamus, thus decreasing the amount of TRH produced, which then signals the anterior pituitary to make less TSH. As a result, less T_3 and T_4 is produced. Comparatively, low levels of T_3 and T_4 signal the hypothalamus and anterior pituitary to increase the levels of TRH and TSH respectively, thus increasing the amounts of T_3 and T_4. A diagnosis of primary hypothyroidism is indicated when the thyroid is not producing sufficient amounts of T_3 and T_4. In the same manner, a diagnosis of secondary hypothyroidism is in order when the anterior pituitary is malfunctioning, thus not adequately producing TSH.

The University of Nebraska Medical Center Hospital and Clinics uses filter paper blood spots for the screening process, which is conducted by nuclear medicine *in vitro*. If the NN T_4 is abnormally low, the same filter paper card is used for a determination of neonatal thyroid-stimulating hormone (NN TSH) to correlate with the low NN T_4. Manufacturers of radioimmunoassay (RIA) kits use either liquid standards or filter paper spot standards in their protocols for assaying for NN T_4 or NN TSH. We prefer the filter paper standards, for this allows consistency between the standards, controls, and patient samples. Additionally, there are no commercially available outside filter paper controls, therefore only kit controls are used with the assays. Heel sticks are performed at three days of age and the blood is soaked into circles on filter paper (Schleicher and Schuell No. 903). It is customary to wait three days, as thyroid hormones are greatly elevated due to the trauma of birth, and they should have returned to their normal levels in 72 hours (*12*). In addition, filter paper samples have the advantages of low cost, reliability, and ease of collection, as venipuncture does not have to be performed (*13,14*). The blood should be taken from the neonate as opposed to cord blood, as maternal thyroid hormones affect cord blood levels substantially (*15,16*).

If both the NN T_4 and NN TSH values are abnormal, then a serum sample is collected and various thyroid RIAs are done, such as T_3, T_3 Uptake, Free T_3, T_4, Free T_4, and TSH. This gives a more complete picture of the child's thyroid status.

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TABLE 1. Evaluation of Neonatal T_4 RIA Kits

Kit	Neometrics		Kallestad		Becton-Dickinson		Nuclear Medical Systems		Meloy		Diagnostic Products	
Run	1	2	1	2	1	2	1	2	1	2	1	2
Percent Bound	46	40	59	60	51	51	68	62	50	37	41	42
Percent Maximum Error	—	—	—	19	—	—	—	14	—	11	—	—
Percent Non-Specific Binding	13.8	14.4	0.15	0.19	0.05	0.04	7.3	7.6	19.4	35.1	0.09	0.10
Slope Value	1.0	0.6	0.9	0.2	1.2	0.8	1.3	0.2	1.0	0.5	0.8	0.9
Y-intercept	2.7	2.3	1.8	0.3	2.9	2.2	3.2	1.0	2.2	1.6	1.6	1.7
Percent Scatter	2	4	2	5	2	3	3	4	3	4	3	2
(Control I) Limits	(1.7–4.5)		(1.99–3.91)		(2.1–5.7)		(2.1–4.1)		(1.97–3.61)		(2.0–5.4)	
Values	3.1	3.3	3.2	3.0	3.9	3.7	5.6	5.2	2.1	2.9	4.0	4.0
(Control II) Limits	(5.6–9.5)		(5.91–10.03)		(10.1–17.4)		(14.5–22.8)		(9.38–14.06)		(5.6–8.6)	
Values	8.1	8.3	8.2	7.6	12.8	13.5	19.9	15.6	10.9	10.0	7.0	6.3
(Control III) Limits	(15.5–24.5)		(11.23–18.31)		———		———		(17.94–25.70)		(16.0–22.2)	
Values	19.4	20.5	13.9	15.1	———		———		18.7	18.4	14.5	14.4
Percent Intra-assay C.V.	5.0	6.8	7.4	7.5	9.7	7.9	10.0	24.2	12.4	10.6	16.2	11.2
Least Detectable Dose	1.0*		0.23		1.05*		——†		1.0*		0.039	
Two-tailed, paired t-test, $p<0.05$	Yes		No		Yes		Yes		Yes		Yes	
Technologist Time	45 min		40 min		50 min		1 hr 20 min		1 hr		50 min	
Total Test Time	21 hr		20 hr		21 hr		22 hr		21 hr		20 hr	
Kit Cost (Cost Per Tube)	$1.00		$0.75		$0.91		$0.80		$0.68		$0.45	

TABLE 2. Evaluation of Neonatal TSH TIA Kits

Kit	Nuclear Medical Systems		Diagnostic Products		Meloy		Becton-Dickinson	
Run	1	2	1	2‡	1	2§	1	2
Percent Bound	29	30	43	11	23	1	18	14
Percent Maximum Error	—	—	22	—	16	13	27	29
Percent Non-Specific Binding	5.5	5.6	7.1	33.6	6.2	24.9	3.5	2.8
Slope Value	0.6	0.7	0.5	0.8	0.9	0.6	0.6	0.5
Y-Intercept	4.0	4.5	3.8	4.9	5.2	4.4	3.3	3.1
Percent Scatter	2	2	6	3	3	4	5	4
(Control I) Limits	(10.7–22.3)		(7.3–15.1)		(21.11–60.19)		(19.1–28.9)	
Values	17.2	16.1	10.4	——	34.4	37.7	16.5	23.3
(Control II) Limits	(124–215)		(38.1–50.1)		(81.49–129.45)		(93.8–114.2)	
Values	129	156	50.9	——	95.7	103	85.1	101
(Control III) Limits	———		(74.5–103.7)		———		———	
Values	———		117	——	———		———	
Percent Intra-assay C.V.	28.1	38.6	42.9	——	42.2	28.7	23.2	44.9
Least Detectable Dose	1.12		1.70		15.5*		2.45	
Two-tailed, paired t-test, $p<0.05$	Yes		———		No		No	
Technologist Time	1 hr 15 min		1 hr 30 min		1 hr 10 min		1 hr	
Total Test Time	26 hr		26 hr		28 hr		25 hr	
Kit Cost (Cost Per Tube)	$0.80		$0.60		$1.44		$0.90	

Footnotes for Tables 1 and 2

* B_0 values were so low they could not be read off of the standard curve. LDD is therefore 95% Bound as read from a Logit-Log, hand-drawn curve.

† B_0 values were so low they could not be read off of the standard curve. The hand-drawn curve on Logit-Log graph paper only reaches 85% Bound, therefore the LDD is not obtainable.

‡ This run could not be completed properly due to experimental error.

§ This run was probably adversely affected by a malfunctioning centrifuge; the refrigeration control broke during this run's centrifugation time.

Materials and Methods

Radioimmunoassay kits evaluated for NN T_4 that use filter paper standards were those made by Becton-Dickinson Immunodiagnostics, Diagnostic Products Corporation, Kallestad Laboratories, Meloy Laboratories, Neometric Incorporated, and Nuclear Medical Systems. The kits evaluated for NN TSH were from Becton-Dickinson Immunodiagnostics, Diagnostic Products Corporation, Meloy Laboratories, and Nuclear Medical Systems. The actual procedures for each kit will not be examined here as such information may be obtained from the manufacturers themselves. Some of the kits offer optional, one-day assays, but all runs were incubated overnight to insure consistency.

Our laboratory uses an automated gamma well counter with an automatic calculator. Two runs performed one week apart were completed on each kit, and various parameters were analyzed. The following criteria were used to evaluate the kits; some parameters have limits, where some do not:

Percent Binding: greater than 25%, where

$$\text{Percent Binding} = \frac{\text{Net Zero Standard } (B_0) \text{ CPM}}{\text{Net Total Count CPM}} \times 100$$

Maximum Error: less than 11%. After the best fit curve is generated, the calculator determines the percent error of the true standard value from the generated curve value. If this error is greater than 5%, then the Non-Specific Binding (NSB) and B_0 CPM are adjusted and the curve is regenerated a maximum of three times. If a greater than 5% error still exists, the calculator prints out a standard curve and maximum error.

Percent NSB: less than 10%, where

$$\text{Percent NSB} = \frac{\text{Net NSB CPM}}{\text{Net Total Count CPM}} \times 100$$

Slope of Curve: should fall within two standard deviations (SD) of the other run of the same kit.

Y-Intercept of Curve: should not considerably differ from the other run of the same kit.

Percent Scatter of Standard Curve: less than 7%. Measures the degree of dispersion of the assayed standards from the best fit curve.

Control Levels: should remain within the specific kit's set limits.

Intra-assay Coefficient of Variation (CV): less than 10%. Using ten blood samples from the same patient,

$$\%\ \text{CV} = \frac{\text{SD}}{\text{mean}} \times 100$$

Least Detectable Dose (LDD): mean − 2 SD. Five samples of B_0 are read from the standard curve, and the mean and SD are calculated from these values.

Parallelism: slopes of diluted and undiluted curves should be within ± 2 SD. This tests the curve response at half each original standard value. A problem was encountered here with some of the kits in accurately obtaining half of a paper punch sample, therefore all of the standards on the parallelism curves for these kits were doubled.

Paired (Student's) t-test: $p < 0.05$. The same neonatal samples were assayed on the duplicate runs from each kit, and two-tailed, paired t-tests were done on the values of each kit to determine if the values were from the same population.

Technologist Time

Total Test Time

Kit Cost (Cost Per Tube)

Company Reliability

The last four parameters are rather subjective and must be weighed by the people performing the assays.

Results and Discussion

The parameters which had been considered to be the most important are percent bound, (absence of) maximum error, percent non-specific binding, percent scatter, control levels, intra-assay coefficient of variation, and student t-test with $p < 0.05$.

The findings from the neonatal thyroxine kit evaluations are summarized in Table 1. Based upon this information, the following three NN T_4 RIA kits best fulfilled the predetermined parameters: Neometric Incorporated, Kallestad Laboratories, and Becton-Dickinson Immunodiagnostics. The Kallestad kit is the least expensive and has the highest percent bound values, but the second run had a maximum error and the t-test was not within the set limit. The Neometric kit had the lowest intra-assay CV, but has comparatively higher percent NSB values and is the most expensive. The Becton-Dickinson kit has extremely low percent NSB values and consistent percent bound values, but has only two kit control levels and is not particularly cost competitive.

Table 2 contains the evaluation results of neonatal thyroid-stimulating hormone kits. The kit which best met the requirements was the one manufactured by Nuclear Medical Systems. This kit is relatively low in cost, has consistent percent bound values, no maximum error, low percent scatter, and is within the set limit on the t-test; however, it has only two kit control levels. Additionally, the intra-assay CV values are not within the predetermined limits, but are low when comparing them with similar values of the other evaluated NN TSH RIA kits.

Two parameters that are not included in Tables 1 and 2 are slope and parallelism. All of the kits fell within ± 2 SD on both slope and parallelism.

Although all parameters were not adequately fulfilled on these kits, they were the most appropriate ones for our use. These findings are not intended to be inclusive of kit quality, as experimental results will always vary; however, these results have been examined in detail as an example of the importance of all of the mentioned parameters when evaluating any type of radioimmunoassay kit.

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