Appendix

To determine how TSH values from the Hanford Thyroid Disease Study in the years 1995-1997 compare to more recent TSH assay methods, frozen sera from 50 subjects were thawed, and divided into two samples. TSH tests were run at 11 am, June 18,2007 using IMX (Abbott Laboratories) on the first sample and ICMA (Bayer Diagnostics) on the second sample. IMX is the available method most similar to the ELISA method that was used for the original HTDS clinic; the comparison between these results was done to assess the stability of TSH on sera that was frozen for 10-13 years, with the additional caveat that it was previously thawed in 1998 to measure anti-TG, and then the remaining sera was refrozen. If under these circumstances the HTDS sera were considered stable, then comparing the original HTDS value to that from ICMA will provide information on how different the HTDS values are from the 3rd generation TSH assays currently in use.

Table 1 summarizes the distributions of TSH values from the three different methods for the 50 subjects. ICMA gave the highest mean value, followed by IMX and then the original HTDS clinic value (ELISA). The correlations of the ICMA and IMX values to the ELISA values were 0.97 and 0.96, respectively (Table 2, Figures 1 and 2).

Table 1. TSH Distribution (μiU/ml) for the three assays (N=50)

	Mean	Std Dev	Median	Min	Max
ICMA	4.01	1.90	3.70	1.23	8.80
IMX	3.43	1.61	3.31	0.99	6.63
ELISA	3.28	1.53	3.26	0.95	6.33

Table 2. Correlation between TSH values for the three assays (N=50)

Pair	Correlation
ICMA & ELISA	0.97
IMX & ELISA	0.96

Regression analyses were performed to estimate the relationships of the IMX or ICMA values (denoted Y) to the original HTDS ELISA values (X), using the simple linear regression equation $Y = \alpha + \beta X + \varepsilon$ where α is the intercept, β is the slope, and ε is the random error. If the two TSH methods gave the same results then the true values of the intercept and slope would be $\alpha=0$ and $\beta=1$. There was one subject for whom the differences between the pairs of TSH values was relatively high and skewed the results of the regressions: the results reported here exclude this outlier (Table 3). For both regression equations the intercept was not significantly different from zero, and for the regression of IMX on ELISA the slope was 0.99, very nearly $\beta=1$. However for the regression of ICMA on ELISA, the slope was significantly greater than 1.

Table 3. Regression of one TSH assay on another excluding 1 outlier (N=49)

Regression model	Intercept (α)	p-value (intercept)[a]	Slope (β)	p-value (slope)[b]
ICMA on ELISA	0.16	0.26	1.16	0.0001
IMX on ELISA	0.18	0.28	0.99	0.77

[a]Test for intercept $\alpha=0$
[b]Test for slope $\beta=1$

As no substantial difference was found when comparing the original TSH values (ELISA) to those from IMX, we concluded the HTDS serum samples did not degrade over time. Thus we then used the regression equation of ICMA = 0.16 + 1.16 x ELISA as the basis for estimating ICMA values. For example, the 97.5th percentile ELISA value of 3.4 μiU/ml for NRG-3 would correspond to an ICMA value of 0..16 + 1.16 x 3.4 = 4.1 μiU/ml.