Design Verification

Total Thyroxine ELISA (T4)

Contents

1 Assay Principle

The test is based on a competitive ELISA technique (microtiter plate) and is used for the quantitative determination of L-thyroxine (T4) in serum. Enzyme-labeled T4 and T4 from the sample compete for binding sites of a sheep anti-T4 antibody coated onto microtiter wells. The more T4 is present in the sample, the less enzyme-labeled T4 is bound. The enzyme activity of the bound T4-enzyme conjugate is photometrically determined by the turnover of TMB substrate.

2 Imprecision

Within-run and between assay imprecisions have been checked by employing pool sera of low, medium and high T4 content. For within-run studies 20-fold determinations have been run and statistically interpreted. For between assay imprecisions data from 10-fold determinations have been evaluated. The results are summarised below.

Within-run Imprecision

Lot 2K2E1

	N	Mean, µg/dl	SD, µg/dl	CV, %
Level 1	22	6.87	0.16	2.3
Level 2	22	9.95	0.16	1.6
Level 3	22	13.1	0.17	1.3

Between run Imprecision

Lot 2K2E1

	N	Mean, µg/dl	SD, µg/dl	CV, %
Level 1	10	2.5	0.19	7.61
Level 2	10	8.6	0.43	5.00
Level 3	10	22.1	1.86	8.42

3 Comparison of Methods

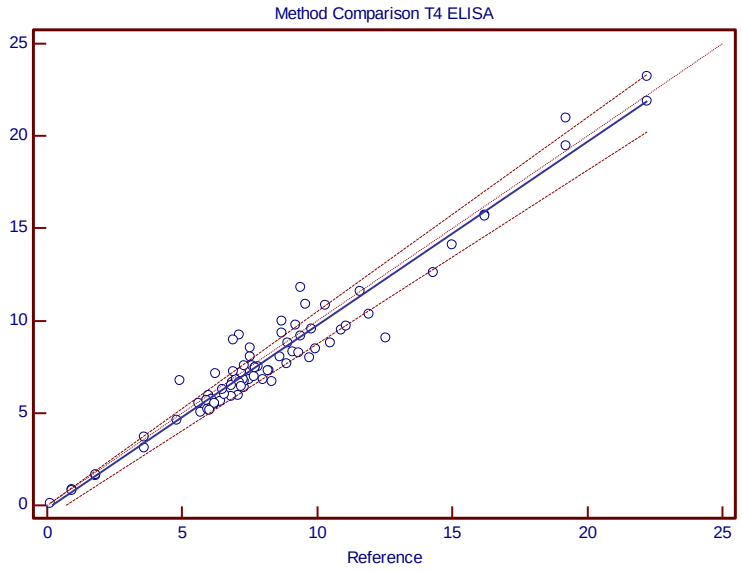
The T4 ELISA test (lot 2K1H0) has been compared against a chemiluminescence immunoassay. 81 specimens from low, normal and high T4 level populations have been tested by an external laboratory employing the competitor's test. The results from the comparison study are summarized below.
The individual data have been evaluated by a non-parametric regression analysis according to Passing & Bablok.

The individual data have been evaluated by a least squares regression analysis as follows:

No. of samples T4 ELISA (Y): 81
No. of samples competitor test (X): 81

Mean Y: 8.05 Median: 7.26
Mean X: 8.32 Median: 7.48

Equation: Y = -0.1874 + X 0.9941 (µg/dl)
r = -0.9398



These data demonstrate the excellent correlation of the HUMAN T4 ELISA to commercially available competitor assays.

4 Linearity

Linearity of the assays was tested by two different methods.

a) Recovery of serial dilution was determined by dilution of a T4 serum with high initial absorbances. As diluent the zero calibrator of the kit was employed.
b) Recovery of spiking of a serum pool with low initial absorbances for T4. The pool was spiked with known amounts of T4 concentrations.

The results (actual recovery vs. calculated result from linear regression) are summarised in the following tables. The percentage in recovery for both methods confirm the excellent linearity of the test

a) Dilution	Recovery µg/dl	Calculated µg/dl	Deviation %
Patient sample	16.12	--	--
1:1	8.24	8.06	2.2
1:4	4.03	4.03	0.0
1:8	2.00	4.02	-0.8

b) Spiking	Recovery µg/dl	Calculated µg/dl	Deviation %
Pool	2.84	--	--
+ 2.0 µg/dl	4.73	4.84	-2.3
+ 4.0 µg/dl	6.62	6.84	-3.3
+ 8.0 µg/dl	10.91	10.84	0.6

5 Sensitivity and Specificity

Sensitivity (Analytical Sensitivity)

The analytical sensitivity was determined by running 24 replicates of T4 zero calibrator in the same assay. The mean and the 2-fold standard deviation of the zero calibrator absorbance was taken and extrapolated from the calibration curve. The sensitivity was determined at 0.22 µg/dl.

Specificity

Potentially crossreacting substances have been added to sera of known predetermined T4 content. The spiked samples have been determined and the results have been compared against the unspiked sera. No crossreactivity could be found with the following substances (reactivity issue: difference in signal ± 10%):

Substance	**Concentration added**
Lot 2K1J4	
d-Thyroxine	10 µg/dl
I-Triiodo-thyronine	100 µg/dl
d-Triodo-thyronine	100 µg/dl
Monoiodo Tyrosine	100 µg/ml
Diiodo-Tyrosine	100 µg/ml
Triiodothyroacetic Acid	100 µg/ml
Tetraiodothyroacetic Acid	100 µg/ml
Albumin	40 mg/ml
Lot 2K3K4	
Salicylate	0.5 mg/ml
Thyroxine Binding Globulin (TBG)	40 µg/ml
Phenytoin	40 µg/ml
Phenylbutazone	10 µg/ml
Hemoglobin	20 µl/ml
Lot 2K1K4	
Lipemia	50 µg/dl

6 Standardisation

For calibration sodium thyroxine pentahydrate is used. It can be obtained in a pure state (confirmed by analytical techniques such as mass spectroscopy and infrared spectrometry) from commercial sources.
Human serum depleted of sodium thyroxine is used as the matrix for preparing the calibrator.
No external standard exists for T4 therefore the assay has been calibrated against accepted commercial kits.

7 Stability

The stability of the T4 ELISA has been evaluated by accelerated temperature stress and real time studies. The results are summarised below.

Accelerated stress (37°C)

The stability of the T4 ELISA has been evaluated by accelerated temperature stress. The results are summarized below.

Lot: 2K2E7

Calibrator	Fresh	Day 3	Day 7	Day 10	Day 20
	OD	OD	OD	OD	OD
A	2.383	2.295	2.282	2.175	2.062
B	1.604	1.642	1.578	1.493	1.351
C	1.172	1.138	1.157	1.094	0.973
D	0.768	0.785	0.759	0.707	0.655
E	0.601	0.581	0.563	0.509	0.432
F	0.367	0.357	0.334	0.314	0.265
	µg/dl	µg/dl	µg/dl	µg/dl	µg/dl
Control 1	2.76	3.52	3.80	4.03	4.11
Control 2	8.28	8.99	9.01	8.76	9.58
Control 3	17.23	16.07	17.45	16.76	21.59

Lot: 2K1B8

Calibrator	Fresh	Day 3	Day 7	Day 10	Day 20
	OD	OD	OD	OD	OD
A	2.440	2.113	2.080	2.063	1.958
B	1.696	1.501	1.478	1.471	1.363
C	1.190	1.093	1.072	1.047	0.988
D	0.805	0.746	0.725	0.685	0.687
E	0.623	0.556	0.545	0.555	0.517
F	0.413	0.342	0.367	0.365	0.337
	µg/dl	µg/dl	µg/dl	µg/dl	µg/dl
Control 1	2.97	3.20	3.10	3.32	2.94
Control 2	8.28	8.76	8.62	8.20	8.95
Control 3	17.90	17.52	19.86	18.49	19.09

Lot: 2K4C8

Calibrator	Fresh	Day 3	Day 7	Day 10	Day 20
	OD	OD	OD	OD	OD
A	2.024	2.004	1.982	1.880	1.765
B	1.345	1.442	1.407	1.330	1.269
C	0.907	0.998	0.992	0.981	0.907
D	0.606	0.625	0.617	0.618	0.601
E	0.472	0.497	0.501	0.467	0.447
F	0.310	0.292	0.305	0.278	0.267
	µg/dl	µg/dl	µg/dl	µg/dl	µg/dl
Control 1	3.08	3.340	3.40	3.37	3.51
Control 2	7.35	8.02	7.88	8.71	8.66
Control 3	16.51	16.12	19.27	17.46	20.83

Real time stability

Three different lots have been stored for 36 months at 2...8°C. The specification are as follows:
After 6 months > 90% recovery of absorbance,
After 12 months > 80% recovery of absorbance,
After 18 months > 70% recovery of absorbance,
After 24 months > 60% recovery of absorbance,
After 36 months > 50% recovery of absorbance,

Lot: 2K1E0

Calibrator	T4 ELISA fresh Time 0		T4 ELISA 12 months	T4 ELISA 18 months	T4 ELISA 24 months	T4 ELISA 36 months
	Concentration	OD	OD	OD	OD	OD
A	0 µg/dl	2.720	2.719	2.451	2.462	2.233
B	2 µg/dl	1.996	2.084	1.859	1.856	1.679
C	5 µg/dl	1.579	1.387	1.309	1.241	1.160
D	10 µg/dl	1.003	0.905	0.842	0.793	0.730
E	15 µg/dl	0.743	0.701	0.614	0.700	0.578
F	25 µg/dl	0.567	0.450	0.405	0.402	0.392
		µg/dl	µg/dl	µg/dl	µg/dl	µg/dl
Control 1		5.66	5.50	5.49	5.49	5.97
Control 2		8.77	8.55	8.19	9.26	9.46
Control 3		13.59	16.62	12.75	19.77	18.21

Lot: 2K3E0

Calibrator	T4 ELISA fresh Time 0		T4 ELISA 12 months	T4 ELISA 18 months	T4 ELISA 24 months	T4 ELISA 36 months
	Concentration	OD	OD	OD	OD	OD
A	0 µg/dl	2.420	2.501	2.236	2.275	2.121
B	2 µg/dl	1.770	1.852	1.661	1.645	1.611
C	5 µg/dl	1.350	1.277	1.176	1.126	1.115
D	10 µg/dl	0.880	0.830	0.761	0.780	0.775
E	15 µg/dl	0.660	0.638	0.577	0.583	0.580
F	25 µg/dl	0.440	0.415	0.370	0.375	0.392
		µg/dl	µg/dl	µg/dl	µg/dl	µg/dl
Control 1		5.38	5.68	5.78	5.38	5.47
Control 2		8.72	9.31	9.69	9.75	9.98
Control 3		14.33	16.92	16.96	15.86	17.47

Lot: 2K6K9

Calibrator	T4 ELISA fresh Time 0		T4 ELISA 12 months	T4 ELISA 18 months	T4 ELISA 24 months	T4 ELISA 36 months
	Concentration	OD	OD	OD	OD	OD
A	0 µg/dl	2.470	2.652	2.637	2.216	2.223
B	2 µg/dl	1.990	2.017	2.153	1.672	1.619
C	5 µg/dl	1.450	1.386	1.395	1.121	1.132
D	10 µg/dl	0.894	0.906	0.935	0.747	0.730
E	15 µg/dl	0.750	0.674	0.652	0.591	0.564
F	25 µg/dl	0.510	0.449	0.400	0.342	0.369
		µg/dl	µg/dl	µg/dl	µg/dl	µg/dl
Control 1		5.44	4.04	4.52	3.83	3.78
Control 2		8.71	6.75	7.35	7.46	7.45
Control 3		17.82	14.80	15.01	16.72	15.90

Conclusion: The results from accelerated and real time stability tests support the claimed shelf life of at least 36 months after production.