Reference Intervals for Children and Adults
Elecsys® Thyroid Tests

TSH, FT4, FT3, T4, T3, T-Uptake, FT4-Index, Anti-TPO, Anti-Tg, Anti-TSHR, Tg, hCT

cobas e analyzers

Table of content

1 Introduction

This brochure is an editorial update (2020) of the highly valued "Reference Intervals for Children and Adults – Elecsys® Thyroid Tests" released in 2018 (material no. 04640292001).

The major changes in this update are;

Removal of the data of Reference population groups N (Norway), A (Austria) and Thailand due to compliancy reasons.

Update of the GEL (children) group data after reanalysis with state-of-the-art method for estimating confidence intervals resulting in updated confidence intervals that are now reported with 95 % confidence level (instead of 90 % confidence level) in line with the representation of the other cohorts.

Additional minor changes in other tables are indicated by the grey change bar in the margin of the page.

The brochure continues to provide reference ranges for different subject groups for all thyroid tests currently on the market, including data for Elecsys® Anti-TSHR, Elecsys® hCT, Elecsys® Tg II.

An overview and description of the reference populations used for the various Thyroid parameter can be found in Chapter 2 "Reference population".

Data from the LIFE study (http://life.uni-leipzig.de) are available, comprising more than 1,000 children between 3 months and 17 years of age and more than 5,300 adults between 18 and 80 years of age. LIFE study data are available for Elecsys® TSH, Elecsys® FT3, Elecsys® FT4, Elecsys® Anti-Tg, Elecsys® Anti-TPO, Elecsys® Anti-TSHR and Elecsys® hCT.

At this moment in time, with respect to the standardization concept, the reference ranges of the currently available thyroid tests are assay generation independent.

In chapter 3 "Statistical Methods" and Chapter 4 "Terminology" biostatistics analysis and background is explained. In subsequent chapters reference ranges for various populations for each thyroid parameter is shown.

The inpatient/outpatient data (previously published under the material number 05968810001) can be found in Chapter 6 starting on page 48.

A one page overview of all populations can be found at the end of the brochure. The overview can be detached and used like a bookmark to support easy orientation.

2 Reference population

2.1 Package insert

In this brochure the reference range data from the package inserts – chapter 2.1 – as well as additional studies – chapter 2.2 – are summarized.

2.1.1 Elecsys® TSH

The reference range population tested for Elecsys® TSH in 1996/97 consisted of 516 euthyroid women and men measured in Vienna, Tokyo, Paris, Tutzing[1].

2.1.2 Elecsys® FT4, Elecsys® T3, Elecsys® T4, Elecsys® T-uptake, FT4-index

The clinical study was conducted in late 1997/early 1998 to introduce a revised Elecsys® T4 test and to validate the reference ranges of the assays, which were established in 1995/1996 at the time of the introduction of the Elecsys® systems or have been adopted from the previous tests – the Enzymun-Test parameters.

The reference population, which is the basis for the information in the current package inserts, consisted of women and men between 25 and 60 years of age, whose serum samples were measured in routine tests in clinical centers in Belgium, Japan and Germany. The groups consisted of blood donors in addition to hospital inpatients and outpatients. The TSH results were within the normal range for the respective routine method for all specimens. No information was available on any medication taken by the subjects. No information on possible pregnancies was available in the female groups. The number of samples, which varied from 370 to 2,526, depending on the test parameter, is given in the tables of the corresponding chapters on the individual tests.

Measurements were taken for Elecsys® FT4, Elecsys® T3, Elecsys® T4 and Elecsys® T-uptake. The FT4 index was determined by the ratio of T4 and T-uptake results.

Reference population USA for T4 and FT4-index: Specimens from 235 serum and plasma samples from healthy test subjects with Elecsys® TSH concentrations within 0.27 to 4.2 µIU/mL and Elecsys® FT4 values within the range of 12.0 to 22.0 pmol/L.

2.1.3 Elecsys® FT3

The reference population tested for Elecsys® FT3, Cat. no. 03051986 (generation taken from the market by now), in 2004 consisted of the group GHH – please see chapter 2.2.

2.1.4 Elecsys® Anti-TPO, Elecsys® Anti-Tg

The reference population tested for Elecsys® Anti-TPO and Elecsys® Anti-Tg in 2000/2001 consisted of women and men whose TSH and FT4 concentrations were within the euthyroid range and the clinical appearance was without any signs for thyroid dysfunction.

Elecsys® Anti-TPO was measured at clinical sites in Germany and Austria.

2.1.5 Elecsys® Anti-TSHR

The reference population tested for Elecsys® Anti-TSHR assay consisted of 436 apparently healthy individuals, 210 patients with thyroid diseases without diagnosis of Graves' disease, and 102 patients with untreated Graves' disease[2].

2.1.6 Elecsys® Tg, Elecsys® Calcitonin

The reference population tested for Elecsys® TgII assay consisted of 478 healthy Caucasian subjects (224 females, 254 males). Measurements were done in Germany.

The reference population tested for Elecsys® Calcitonin assay consisted of 355 German apparently healthy subjects (193 females, 162 male) and 364 US apparently healthy subjects (180 female, 184 male).

2 Reference population

2.2 Reference population groups

2.2.1 Group GEL

Newborns, infants, children and adolescents in age 0 – 20 years from Erlangen and Leipzig, Germany 2003, 2004, 2007 (Group GEL)

The reference range calculation for the thyroid parameters was made using sample material (serum and plasma collected in Erlangen and Leipzig from 2002 to 2004 and also in Leipzig from 1990 to 2010). This combined collective is referred to as Group GEL in the brochure. In the subgroup Erlangen a total of approximately 400 specimens and in the subgroup Leipzig a total of approximately 1,000 specimens of newborns, children and adolescents of both sexes and in the age of 0 and <20 was included. To gain a better statistical computation for the reference ranges the whole Group GL was included. Reference intervals were only reported till the age of 20 years. Depending on the parameter between 47 – 473 specimens in each of the GEL subgroups are shown.

Measurements were taken for Elecsys® TSH, Elecsys® FT4, Elecsys® FT3, Elecsys® T3, Elecsys® T4, Elecsys® T-uptake, Elecsys® Anti-TPO and Elecsys® Anti-Tg. The fT4-index was determined from the T4 and T-uptake (TBI) ratio.

2.2.2 Group GL

Adults, Leipzig, Germany 2003 and 2004

To determine the reference ranges of thyroid parameters, in 2003/2004, at the "Universitätsklinikum Leipzig", Germany, serum specimens were used from a total of 870 blood donors between the ages of 18 and 60 years.

The following information is available for these specimens: age, sex, height, weight, personal and family history of thyroid disorders, contraceptive use and cigarette use. Measurement results are available for: thyroid volume and structure, blood pressure, heart rate, blood count, lipid metabolism parameters, protein, albumin and the thyroid parameters including autoantibodies.

The TSH inclusion or exclusion criteria were ensured based on the results of a commercially available test (ADVIA Centaur, Bayer, Germany).

The group GL equals GL1. Furthermore, the groups GL2, GL3, GL5 have been derived from GL1 by applying different inclusion and exclusion criteria. A summary is depicted in the table below.

Group GL1
Blood donors, all men and women: all tested blood donors, no in- and exclusion criteria
Group GL2
Inclusion criteria: normal thyroid ultrasound (volume and structure), ADVIA Centaur TSH within reference range >0.35 and <5.5 µIU/mL
Group GL3
Inclusion and exclusion criteria according to Guideline 22 of the National Academy of Clinical Biochemistry (NACB), USA 2002 – Recommendations on determining reference intervals of TSH[3] Inclusion criteria: no visible or palpable goiter (ultrasound normal, structure and volume of thyroid normal), Elecsys® Anti-TPO (<34 IU/mL) and Elecsys® Anti-Tg (<115 IU/mL) determined from Elecsys® results. Exclusion criteria: subjects with personal or family history of thyroid dysfunction, no medications (except contraceptives).
Group GL5
Inclusion and exclusion criteria according to Guideline 33 of the NACB, USA 2002 – Recommendations on determining reference intervals of Anti-TPO and Anti-Tg.[3] Inclusion criteria: only male samples, non-smoker, ADVIA Centaur TSH within range >0.5 and <2.0 µIU/mL, age <30 years, normal thyroid ultrasound (volume and structure), no non-thyroid autoimmune diseases (e.g. lupus erythemathodes or diabetes). Elecsys® Anti-TPO (<34 IU/mL) and Elecsys® Anti-Tg (<115 IU/mL). Exclusion criteria: subjects with personal or family history of thyroid dysfunction excluded.

2.2.3 Group P

Pregnant women in their 1st, 2nd and 3rd trimester of pregnancy

The group consists of 957 samples of healthy, pregnant women from Essen (436) and 521 samples of healthy, pregnant women (<40 years) from Hamburg with a normal pregnancy without known complications.

Exclusion criteria for this group were: A known or supposed thyroid dysfunction or history, substitution of thyroid hormones, Elecsys® FT3 concentrations <3 pmol/L and Elecsys® FT4 concentrations >22 pmol/L. Samples with Elecsys® TSH concentrations outside a range of 0.1 to 10 µIU/mL were excluded in both groups.

2.2.4 LIFE cohort

Adults and children, Leipzig, Germany 2011 – 2016

The life study is a project of the university Leipzig (www.life.uni-leipzig.de). Goal of the study is to investigate diseases of the civilized population like depression, diabetes, allergy etc. For this purpose, as many information as possible about the health and environment of the general population is collected. Adults are invited once to fill out a questionnaire and undergo physical examination while children are invited several times to participate in the study and follow up their development over time.

The data of the LIFE study have been provided to Roche Diagnostics to determine reference ranges. Inclusion/exclusion criteria for adults and children differ, therefore, they are split into two groups. As children might have participated more than once, they have been considered to be apparently thyroid healthy only if the inclusion criteria have been fulfilled at every visit.

LIFE adult (Age: <18 to >81 years)
Elecsys® TSH, Elecsys® FT4, Elecsys® FT3 Inclusion criteria: no known thyroid disease, Elecsys® Anti-TPO <34 IU/mL and Elecsys® Anti-TSHR <1.75 IU/mL, no medication with T4, iodine substitution, thyreostatic drugs, T3/T4 compounds Exclusion criteria: albumin measuring results out of reference range (adults 39.7 – 49.4 g/L measured on Clinical Chemistry module on **cobas®** 6000/8000
Elecsys® Anti-TPO and Elecsys® Anti-Tg Inclucion criteria: no known thyroid disease, no medication with T4, iodine substitution, thyreostatic drugs, T3/T4 compounds Exclusion criteria: albumin measuring results out of reference range (adults 39.7 – 49.4 g/L measured on Clinical Chemistry module on **cobas®** 6000/8000
Elecsys® Anti-TSHR Inclusion criteria: no known thyroid disease, Elecsys® Anti-TPO <34 IU/mL, Elecsys® Anti-TG <115 IU/mL, no medication with T4, iodine substitution, thyreostatic drugs, T3/T4 compounds Exclusion criteria: albumin measuring results out of reference range (adults 39.7 – 49.4 g/L measured on Clinical Chemistry module on **cobas®** 6000/8000. Lower measuring range (MR) for study was set to <0.90.
The following criteria have been applied to LIFE adult patients to additionally filter for increased BMI: All healthy, male, female patients (BMI >30 and increased waist to hip ratio, female ≥0.85, male ≥0.9). Body mass index (BMI) was calculated using the formula: BMI = weight [kg] / height^2 [m]^2. Waist-Hip ratio was calculated using the formula: waist [cm]/hip [cm].

LIFE child (Age: 3 month to >18 years)
Elecsys® TSH, Elecsys® FT4, Elecsys® FT3, Elecsys® Calcitonin Inclusion criteria: Elecsys® Anti-TPO <34 IU/mL, Elecsys® Anti-TSHR <1.75 IU/mL, Elecsys® Anti-TG <115 IU/mL, no thyroid disease, thyroid ultrasound normal, no medication except for vitamin D supplementation and contraceptives, no known endocrine disease, no reported stationary stay in hospital
Elecsys® Anti-TPO and Elecsys® Anti-Tg Inclusion criteria: no thyroid disease, thyroid ultrasound normal, no medication except for vitamin D supplementation and contraceptives, no known endocrine disease, no reported stationary stay in hospital
Elecsys® Anti-TSHR Inclusion criteria: Elecsys® Anti-TPO <34 IU/mL, Elecsys® Anti-TG <115 IU/mL, no thyroid disease, thyroid ultrasound normal, no medication except for vitamin D supplementation and contraceptives, no known endocrine disease, no reported stationary stay in hospital. Lower measuring range (MR) for study was set to <0.30.

2.2.5 Local reference ranges

A local reference range for Elecsys® FT4 was determined in a clinical center in Germany, which has kindly been provided to us for publication.

Group GHH
Hamburg, Germany, population for Elecsys® FT3 and Elecsys® FT4, n=5,366 specimens came from a large commercial community laboratory. Inclusion criteria: TSH concentration from 1– 3 mIU/L, no other information on the patients was analyzed. This corresponds to a so called “routine collective” as it has been also used for earlier reference interval determinations.

3 Statistical methods

3.1 Computation of quantiles

Quantiles are suitable methods to describe the scattering of data. The clinical and laboratory standards institute (CLSI) guidelines recommend the calculation of the 2.5 % and 97.5 % quantiles for reference ranges (also called reference intervals), see CLSI C28-A3c[5].

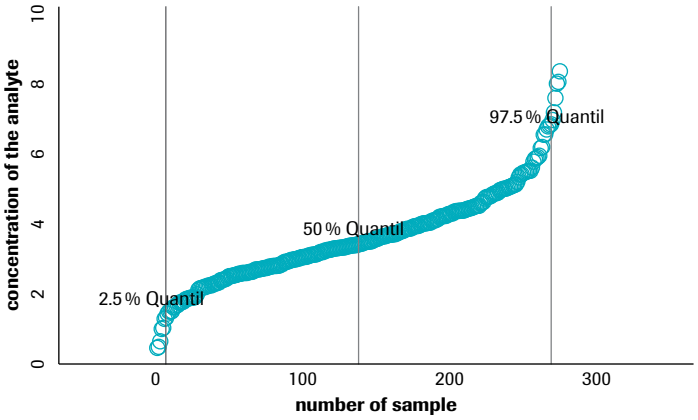


Figure 1: Graphical illustration of the determination of quantiles. The measured samples are presented in ascending order. The vertical lines describe the 2.5 %, 50 % and 97.5 % quantile, respectively.

The central 95 % reference range corresponds to the 2.5 % and 97.5 % quantiles. Figure 1 illustrates the distribution of measured results from a routine cohort. Looking at the area around the 2.5 % and 97.5 % quantile, there are only a few measured values. The fewer data points are found at the outer ends, the less reliable is the biostatistics computation.

The recommendation of CLSI C28-A3c is the use of the 95 % reference range. For every laboratory it is very highly recommended to determine own reference ranges.

Instruction to compute reference ranges

Generally, reference ranges have to be computed for the population of interest. In most cases this corresponds to an apparently healthy population. The correct inclusion and exclusion criteria have to be chosen thoughtfully. The size of the population should be at least 120 samples to calculate the central 95 % interval with confidence level of 90 for the 2.5 % and 97.5 quantile. With higher number of well-chosen samples, the estimation of the reference ranges improves.

One possible way to compute quantiles is the following (see also figure 2):

1. Healthy population containing n measured values
2. Sort measured values in increasing order
3. Apply the following formula: n (number of measured samples) × percentile = position in list

Sample no.	Measured value (pIU/mL)	
1	1.18	
2	1.24	
3	1.27	**2.5th percentile (0.025 ×120 = 3)**
4	1.29	
5	1.33	
6	1.34	
…	…	**N ×percentile = position in list**
114	4.61	
115	4.76	
116	5.13	
117	5.20	**97.5th percentile (0.975 ×120 = 117)**
118	5.31	
119	5.44	
120	5.45	

Figure 2: exemplary table for computing quantiles. The 2.5 % quantile corresponds to sample number on position 3 if n = 120 samples (0.025 × 120), 97.5 % quantile corresponds to sample number on position 117 if n = 120 samples (0.975 × 120). In this example the 95 % reference interval ranges from 1.27 – 5.20 µIU/mL.

3 Statistical methods

3.2 Quantile regression

If reference ranges for target variables are influenced by covariates like age or sex, quantile regression is an appropriate method for calculation. Goal of the quantile regression is the evaluation of the correlation between covariate and target variable for a given quantile.

Quantile regression is a nonparametric method to estimate covariate dependent quantiles. Hence no distributional properties have to be verified. For the quantile regression the same quantiles like recommended by the CLSI C28-A3c are computed (2.5 %, 97.5 % quantile).

For LIFE child the quantile regression has been computed for every parameter over the age from 3 months to 20 years and age segmentation has been performed out of this computation.

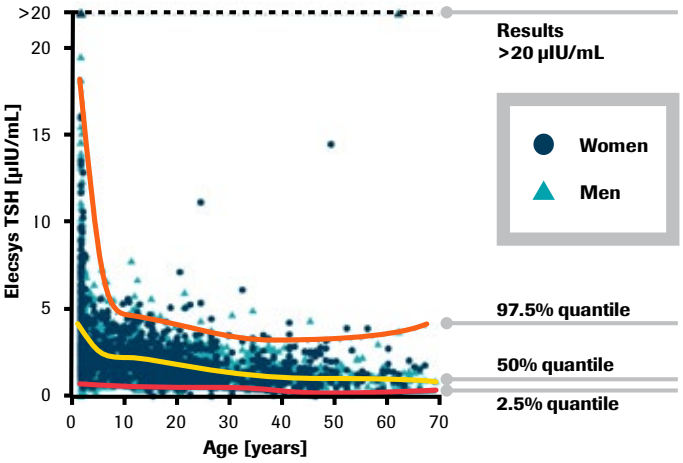


Figure 3: Exemplary quantile regression graphic in which the dependency of TSH in terms of age is depicted.

3.3 Boxplot

Boxplot is a graphical way to describe the distribution of one parameter. The illustration shows different robust measures of dispersion. 50 % of the data are represented in a box defined by the 25 % and 75 % quantile. In addition, the location of the median is described.

The interquartile range (IQR) is defined as the difference of the 75 % quantile minus 25 % quantile. The above whisker is computed as follows: 75 % quantile plus 1.5 × IQR. And accordingly the below whisker is computed by 25 % quantile minus 1.5 × IQR. Both whiskers do not always end at exact that length, but at the nearest value inside the 1.5 × IQR limits.

Boxplots are very suitable methods to compare different groups with each other indicating the median, 25 % and 75 % quantile and the whiskers and thereby visualizing differences at a glance.

Figure 4: Description of boxplot.

3 Statistical methods

3.4 Statistical Programming

Quantiles were computed with the statistical programming language R (version 3.2.1), particulary the function quantile (type=4).

Quantile regression was computed with the function gcrq() from the R package quantregGrowth in R. Similar analyses are described by Kristin Rieger et al. 2016.[6]

Minor changes in results to the previous version of the brochure "Reference Intervals for Children and Adults" (material no. 04640292001) might appear due to the use of different software versions or different calculation software. Former computations with the software SAS were done with the version 9.1 (or 9.1.3). Actual computations were done with the version 9.3. New analyses were done with the statistical programming language R (version 3.2.1). This change can lead to differences in the computation because R and SAS use different methods to calculate quantiles and confidence intervals.

4 Terminology

Reference population/reference group:
Random samples from the population of asymptomatic patients. The reference population provides the basis for determining reference intervals. The reference population is also called reference group.

Reference ranges/reference intervals/reference values/ expected values:
In accordance with IFCC recommendations, the reference intervals or reference ranges are the non-parametrically estimated 2.5 % and 97.5 % quantiles (= percentiles) of the reference population. The 2.5 % quantile designates the value for which 2.5 % of the data are smaller or equal than this value; the 97.5 % quantile designates the value for which 97.5 % of the data are smaller or equal than the quantile value. Occasionally, the reference values are also called reference limits because they bracket the reference range. Reference ranges are sometimes also referred to as 95 % central range (e.g. 2.5 % of all data are below and above this range).

Elecsys® package inserts contain the term expected values instead of reference values or reference intervals.

Confidence interval (CI):
In the given context, the range around the estimated quantile, which includes the true value of the quantile with a given coverage probability. The confidence interval decreases in size as the sample size increases. Figure 5 shows the position of the 95 % confidence intervals of the 2.5 %, the 50 % (median) and 97.5 % quantile.

Coverage probability:
Probability (typically a value of 95 % is selected if possible) that the true quantile value is covered by the confidence interval. Samples that are too small lead to insufficient coverage probabilities. Note that the IFCC recommends at least n = 120 to compute confidence intervals with 90 % coverage probability.

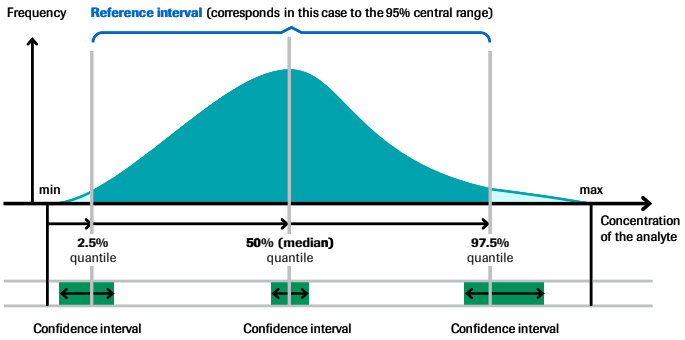


Figure 5: Description of reference range

The tables in this brochure are structured as illustrated in figure 5.

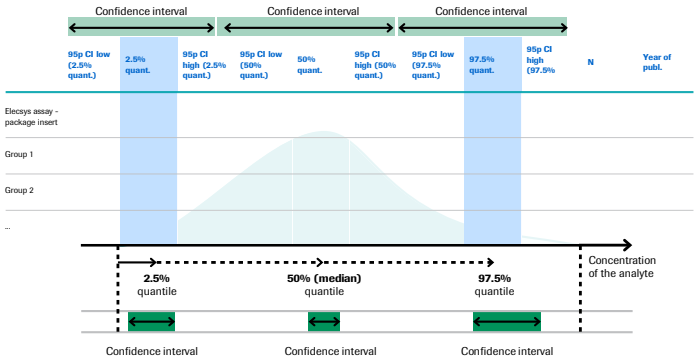


Figure 6: The investigated group is described in the first column. In the last two columns the size of the group (N) and the year of publication are stated. Column 2 – 4 describe the 2.5 % quantile with the lower and upper confidence interval (CI). Column 5 – 7 describe the median 50 % quantile with the lower and upper CI. Column 8 – 10 describe the 97.5 % quantile with the lower and upper CI.

5 Results and Discussion

5.1 Thyrotropin, Thyroid stimulating hormone – TSH

3rd generation TSH tests (defined with functional sensitivity of 0.01 – 0.02 mIU/L) are used as first line parameters to identify thyroid dysfunction. Even very slight changes in the concentrations of the free thyroid hormones bring about much greater opposite changes in the TSH level. Accordingly, TSH is a very sensitive and specific parameter for assessing thyroid function and is particularly suitable for early detection or exclusion of disorders in the central regulating circuit between the hypothalamus, pituitary and thyroid[7-11].

The tables 1 to 4 below describe the TSH reference ranges determined in different studies for: 1. adults, separated into male and female and age, 2. pregnant women, separated into the 3 trimesters and 3. children, separated by age. All confidence intervals were computed with 95 % confidence level.

① TSH	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
					µIU/mL						
Elecsys® TSH – package insert		0.27						4.2		516	96/97
Group GL1 (all blood donors)	0.21	0.30	0.37	1.25	1.31	1.35	3.35	3.69	4.32	869	2004
L1 Males all	0.24	0.30	0.40	1.21	1.25	1.32	3.01	3.18	4.32	445	2004
L1 Males 20 – 39 y	0.29	0.46	0.58	1.32	1.42	1.55	3.04	3.25	4.66	286	2004
L1 Males 40 – 69 y	0.18	0.21	0.30	0.87	0.96	1.07	2.09	2.52	4.32	150	2004
L1 Females all	0.12	0.30	0.39	1.31	1.38	1.47	3.54	3.94	7.18	424	2004
L1 Females 20 – 39 y	0.10	0.44	0.58	1.35	1.46	1.58	3.36	3.63	6.15	269	2004
L1 Females 40 – 69 y	0.05	0.16	0.37	1.01	1.21	1.37	2.86	3.94	66.8	128	2004
L1 Females with contracept.	0.10	0.39	0.60	1.34	1.46	1.59	3.36	3.82	11.2	236	2004
L1 Females with contracept. 20 – 39 y	0.10	0.52	0.62	1.38	1.51	1.60	3.49	5.09	11.2	181	2004
L1 Females with contracept. 40 – 69 y	0.30	0.30	0.37	0.75	1.20	1.59	2.68	2.88	2.88	33	2004
L1 Females w/o contracept	0.05	0.16	0.37	1.18	1.30	1.42	3.22	4.25	66.8	187	2004
L1 Females w/o contracept. 20 – 39 y	0.05	0.22	0.60	1.18	1.36	1.67	2.80	3.05	4.47	87	2004
L1 Females w/o contracept. 40 – 69 y	0.05	0.14	0.37	1.01	1.27	1.42	3.22	4.25	66.8	95	2004
Group GL2 (TSH + SD-Sono NAD)	0.54	0.60	0.65	1.36	1.44	1.52	3.35	3.69	4.32	631	2004
L2 Males	0.52	0.58	0.64	1.29	1.37	1.46	3.04	3.44	4.66	332	2004
L2 Females	0.51	0.63	0.72	1.40	1.52	1.64	3.49	3.82	5.29	299	2004
Group GL3 org (NACB crit. for TSH)	0.29	0.40	0.47	1.29	1.36	1.44	3.14	3.77	4.47	447	2004
L3 Males	0.20	0.36	0.46	1.24	1.34	1.43	3.01	3.44	5.12	274	2004
L3 Females	0.12	0.44	0.63	1.30	1.42	1.59	3.14	3.94	5.29	173	2004
LIFE Adults	0.41	0.45	0.47	1.46	1.49	1.51	3.77	3.91	4.09	5,482	2013
all male patients (sd healthy)	0.38	0.41	0.46	1.40	1.43	1.47	3.59	3.74	3.98	3,169	2013
male <40 years	0.49	0.81	0.98	1.78	1.92	2.08	3.92	4.33	6.17	156	2013
male ≥40 and <70 years	0.39	0.45	0.48	1.39	1.42	1.46	3.47	3.67	3.86	2,322	2013
male ≥70 years	0.22	0.31	0.38	1.27	1.35	1.42	3.30	3.83	4.49	691	2013
all female patients (sd healthy)	0.46	0.48	0.54	1.52	1.55	1.60	3.88	4.13	4.30	2,313	2013
female <40 years	0.16	0.50	0.86	1.73	1.91	2.23	4.57	5.10	7.25	155	2013
female ≥40 and <70 years	0.48	0.54	0.58	1.53	1.57	1.62	3.86	4.07	4.31	1,760	2013
female ≥70 years	0.20	0.28	0.43	1.27	1.38	1.44	3.06	3.45	3.86	398	2013

② TSH	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	µIU/mL										
Pregnant Women											
1st Trimester	0.21	0.33	0.48	1.37	1.48	1.61	3.99	4.59	5.80	418	2004
2nd Trimester	0.21	0.35	0.41	1.38	1.52	1.60	3.61	4.10	4.80	369	2004
3rd Trimester	0.11	0.21	0.29	1.32	1.42	1.59	2.78	3.15	7.08	170	2004

③ TSH	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	µIU/mL										
Group GEL Children, Adolescents											
0 – 6 Days	0.40	0.70	1.28	4.30	4.74	5.25	16.2	20.0	22.8	113	2007
>6 Days ≤3 Months	0.49	0.72	1.04	3.61	3.85	4.10	11.2	12.7	14.1	121	2007
>3 ≤12 Months	0.57	0.73	0.91	3.11	3.25	3.40	8.26	8.92	9.74	123	2007
>1 ≤6 Years	0.57	0.69	0.80	2.49	2.57	2.67	5.65	5.89	6.32	346	2007
>6 ≤11 Years	0.48	0.60	0.68	2.06	2.12	2.20	4.47	4.66	5.04	265	2007
>11 ≤20 Years	0.42	0.51	0.59	1.79	1.84	1.90	4.01	4.17	4.48	471	2007

④ TSH	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	µIU/mL										
LIFE Child											
>3 ≤12 Months	0.85	1.02	1.27	2.94	3.24	3.48	5.92	6.75	8.07	90	2016
>1 ≤6 Years	0.87	1.09	1.20	2.51	2.70	2.85	5.57	6.07	6.80	200	2016
>6 ≤11 Years	1.04	1.13	1.24	2.29	2.41	2.61	4.95	5.34	5.82	250	2016
>11 ≤20 Years	0.97	1.01	1.12	2.06	2.25	2.42	4.58	5.09	5.39	208	2016

The selection of inclusion and exclusion criteria clearly influences the width of the range. This is also reflected by the different groups GL1, GL2, GL3, LIFE adult and LIFE child. In general: the stricter the inclusion and exclusion criteria, the slimmer the reference range.

The TSH reference ranges show a clear age dependency (see figure 7).

Over trimester 1 to 3, pregnant women show a tendency in the upper limits towards lower TSH concentrations. As also recommended in the guidelines from the American Thyroid Association, a trimester specific reference range for TSH shall be applied.[12]

The group LIFE adult did not reveal any dependencies on BMI nor >30 neither >35 with a hip/waist ratio according the WHO. Furthermore, the reference ranges determined for healthy non-smokers, healthy former smokers and healthy smokers are comparable to each other.

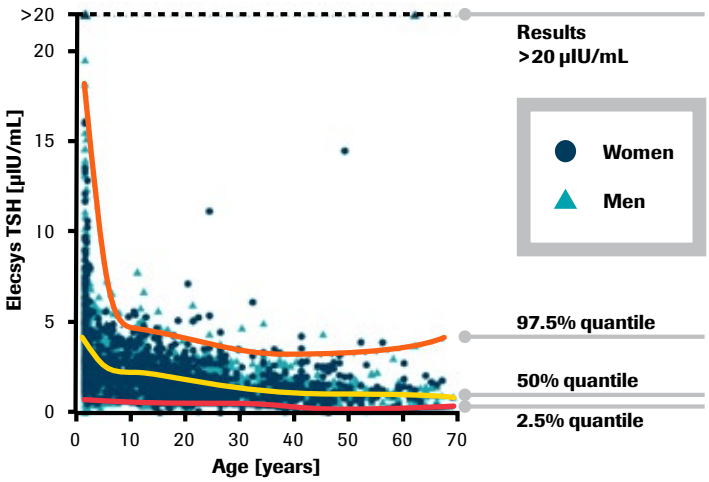


Figure 7: Containing data from GL1 and GEL, shows measured data for children and adults from birth to 70 years of age using separate symbols to distinguish between male and female. The TSH concentration is high in the first days after birth and continuously decreases with age. This emphasizes the importance of age-specific reference ranges for TSH.

5 Results and Discussion

5.2 Free thyroxin – FT4

The determination of free T4 has the advantage of being independent of changes in the concentrations and binding properties of the binding proteins; additional determination of a binding parameter (T-uptake, TBG) is therefore unnecessary. Free T4 is a useful tool in clinical routine diagnostics for the assessment of the thyroid status. It should be measured together with TSH if thyroid disorders are suspected and is also suitable for monitoring thyrosuppressive therapy.[13]

The tables 5 to 12 below describe the Elecsys® FT4 reference ranges determined in different studies for: 1. adults, separated into male and female and age, 2. pregnant women, separated into the 3 trimesters and 3. children, separated by age. All confidence intervals were computed with 95 % confidence level.

5 FT4	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
					pmol/L						
Elecsys® FT4 – package insert		12						22		801	1998
Group GL1 (all blood donors)	12.3	12.7	13.0	16.0	16.2	16.5	20.4	20.8	21.4	870	2004
L1 Males all	12.8	13.1	13.4	16.7	16.9	17.2	20.8	21.3	22.2	445	2004
L1 Males 20 – 39 y	12.9	13.4	14.0	16.9	17.2	17.5	20.7	21.3	23.0	286	2004
L1 Males 40 – 69 y	10.3	12.5	13.2	15.8	16.2	16.8	19.9	21.2	22.2	150	2004
L1 Females all	11.5	12.3	12.7	15.4	15.6	15.9	20.0	20.2	21.0	425	2004
L1 Females 20 – 39 y	11.7	12.4	12.9	15.4	15.7	16.0	19.9	20.4	21.7	270	2004
L1 Females 40 – 69 y	8.24	11.5	13.3	15.2	15.6	15.9	18.9	19.6	21.0	128	2004
L1 Females with contracept.	11.5	12.4	12.8	15.3	15.6	16.0	19.6	20.0	21.7	236	2004
L1 Females with contracept. 20 – 39 y	11.5	12.4	12.8	15.3	15.7	16.1	19.4	19.9	21.7	181	2004
L1 Females with contracept. 40 – 69 y	12.3	12.3	13.2	14.1	14.9	16.1	17.1	19.0	19.0	33	2004
L1 Females w/o contracept	8.24	11.7	13.3	15.4	15.7	15.9	20.0	20.4	28.6	188	2004
L1 Females w/o contracept. 20 – 39 y	11.7	13.0	13.6	15.3	15.7	16.7	20.0	20.5	28.6	88	2004
L1 Females w/o contracept. 40 – 69 y	8.24	9.30	13.4	15.3	15.7	16.0	19.0	20.1	21.0	95	2004
Group GL2 (TSH + SD-Sono NAD)	12.3	12.8	13.0	15.9	16.2	16.5	20.2	20.7	21.4	632	2004
L2 Males	12.7	13.1	13.4	16.7	17.0	17.3	20.4	21.3	23.0	332	2004
L2 Females	11.5	12.3	12.8	15.3	15.6	15.8	19.6	20.1	20.8	300	2004
Group GL3 org (NACB crit. for TSH)	12.2	12.8	13.0	15.9	16.2	16.6	20.2	20.4	21.5	448	2004
L3 Males	10.3	12.9	13.2	16.7	17.0	17.4	20.4	21.4	24.7	274	2004
L3 Females	9.30	12.2	12.8	15.3	15.5	15.8	19.2	20.0	20.7	174	2004
LIFE Adults	11.8	11.9	12.1	15.6	15.7	15.7	19.9	20.1	20.3	5,476	2013
all male patients (sd healthy)	11.8	12.0	12.2	15.7	15.8	15.9	19.9	20.2	20.5	3,169	2013
male <40 years	9.56	12.9	13.82	16.0	16.2	16.6	19.9	21.4	22.5	156	2013
male ≥40 and <70 years	11.7	11.9	12.2	15.7	15.8	15.9	19.8	20.2	20.5	2,323	2013
male ≥70 years	11.6	12.1	12.3	15.7	15.8	16.0	19.8	20.2	21.0	690	2013
all female patients (sd healthy)	11.7	11.8	12.0	15.4	15.5	15.6	19.5	20.0	20.3	2,307	2013
female <40 years	10.6	11.2	11.9	14.7	15.2	15.7	18.9	19.9	21.2	155	2013
female ≥40 and <70 years	11.6	11.8	12.0	15.3	15.4	15.5	19.5	19.8	20.4	1,754	2013
female ≥70 years	11.6	12.2	12.7	15.9	16.1	16.3	19.5	20.2	23.2	398	2013
Results from various locations											
Group GHH (routine samples)	11.2	11.4	11.5		15.7		21.7	22.0	22.4	5,365	2004

⑥ FT4	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	pmol/L										
Pregnant Women											
1st Trimester	11.8	12.1	12.4	15.1	15.4	15.7	19.2	19.6	21.4	418	2004
2nd Trimester	9.18	9.63	10.0	12.6	12.9	13.1	16.1	17.0	18.0	369	2004
3rd Trimester	6.95	8.39	9.31	11.6	11.9	12.3	14.8	16.0	17.8	169	2004

⑦ FT4	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	pmol/L										
Group GEL Children, Adolescents											
0 – 6 Days	8.93	11.0	12.5	17.2	17.8	18.7	29.5	32.1	34.7	111	2007
>6 Days ≤3 Months	9.83	11.5	12.5	17.1	17.4	18.0	26.8	28.4	30.1	113	2007
>3 ≤12 Months	10.6	11.9	12.5	17.0	17.2	17.5	24.6	25.7	26.9	117	2007
>1 ≤6 Years	11.7	12.3	12.6	16.7	16.9	17.0	22.1	22.8	23.6	344	2007
>6 ≤11 Years	12.1	12.5	12.7	16.4	16.6	16.8	20.9	21.5	22.1	263	2007
>11 ≤20 Years	12.2	12.6	12.8	16.3	16.5	16.6	20.5	21.0	21.6	469	2007

⑧ FT4	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	pmol/L										
LIFE Child											
>3 ≤12 Months	12.6	13.3	13.8	15.9	16.2	16.7	19.2	20.5	21	83	2016
>1 ≤6 Years	12.9	13.4	13.7	15.9	16.3	16.5	19.6	20.1	20.6	193	2016
>6 ≤11 Years	12.4	12.9	13.1	15.7	15.9	16.2	19.3	19.7	20.4	243	2016
>11 ≤20 Years	11.2	11.6	12.0	14.9	15.3	15.6	19.3	19.6	20.8	206	2016

9 FT4	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
					ng/dL						
Elecsys® FT4 – package insert		0.93						1.71		801	1998
Group GL1 (all blood donors)	0.95	0.99	1.01	1.24	1.26	1.28	1.58	1.62	1.67	870	2004
L1 Males all	0.99	1.01	1.04	1.30	1.31	1.34	1.62	1.65	1.72	445	2004
L1 Males 20 – 39 y	1.00	1.04	1.09	1.31	1.33	1.36	1.61	1.65	1.79	286	2004
L1 Males 40 – 69 y	0.80	0.97	1.02	1.23	1.25	1.30	1.55	1.65	1.72	150	2004
L1 Females all	0.89	0.95	0.99	1.20	1.21	1.23	1.55	1.57	1.63	425	2004
L1 Females 20 – 39 y	0.91	0.97	1.00	1.20	1.22	1.25	1.55	1.58	1.68	270	2004
L1 Females 40 – 69 y	0.64	0.89	1.03	1.18	1.21	1.24	1.47	1.52	1.63	128	2004
L1 Females with contracept.	0.90	0.97	0.99	1.19	1.21	1.24	1.52	1.56	1.68	236	2004
L1 Females with contracept. 20 – 39 y	0.90	0.97	0.99	1.19	1.22	1.25	1.51	1.55	1.68	181	2004
L1 Females with contracept. 40 – 69 y	0.95	0.95	1.03	1.10	1.16	1.25	1.33	1.48	1.48	33	2004
L1 Females w/o contracept	0.64	0.91	1.03	1.20	1.22	1.24	1.55	1.58	2.22	188	2004
L1 Females w/o contracept. 20 – 39 y	0.91	1.01	1.05	1.19	1.22	1.30	1.55	1.59	2.22	88	2004
L1 Females w/o contracept. 40 – 69 y	0.64	0.72	1.04	1.18	1.22	1.25	1.47	1.56	1.63	95	2004
Group GL2 (TSH + SD-Sono NAD)	0.95	0.99	1.01	1.23	1.26	1.28	1.57	1.61	1.67	632	2004
L2 Males	0.99	1.01	1.04	1.30	1.32	1.34	1.59	1.65	1.79	332	2004
L2 Females	0.90	0.95	0.99	1.19	1.21	1.23	1.52	1.56	1.62	300	2004
Group GL3 org (NACB crit. for TSH)	0.94	0.99	1.01	1.23	1.26	1.29	1.57	1.59	1.67	448	2004
L3 Males	0.80	1.00	1.02	1.30	1.32	1.35	1.58	1.67	1.92	274	2004
L3 Females	0.72	0.94	0.99	1.19	1.20	1.23	1.49	1.56	1.61	174	2004
LIFE Adults	0.92	0.93	0.94	1.21	1.22	1.22	1.54	1.56	1.58	5,476	2013
all male patients (sd healthy)	0.91	0.93	0.95	1.22	1.23	1.24	1.55	1.57	1.59	3,169	2013
male <40 years	0.74	1.00	1.07	1.24	1.26	1.29	1.55	1.67	1.75	156	2013
male ≥40 and <70 years	0.91	0.93	0.95	1.22	1.22	1.23	1.54	1.57	1.6	2,323	2013
male ≥70 years	0.90	0.94	0.95	1.22	1.23	1.24	1.54	1.57	1.63	690	2013
all female patients (sd healthy)	0.91	0.92	0.93	1.20	1.20	1.21	1.52	1.55	1.58	2,307	2013
female <40 years	0.82	0.87	0.93	1.14	1.18	1.22	1.47	1.54	1.64	155	2013
female ≥40 and <70 years	0.90	0.92	0.93	1.19	1.20	1.21	1.51	1.53	1.58	1,754	2013
female ≥70 years	0.90	0.95	0.98	1.23	1.25	1.26	1.52	1.57	1.80	398	2013
Results from various locations											
Group GHH (routine samples)	0.87	0.89	0.90		1.22		1.69	1.71	1.74	5,365	2004

(10) FT4	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	ng/dL										
Pregnant Women											
1st Trimester	0.92	0.94	0.96	1.17	1.20	1.22	1.49	1.52	1.66	418	2004
2nd Trimester	0.71	0.75	0.78	0.98	1.00	1.02	1.25	1.32	1.40	369	2004
3rd Trimester	0.54	0.65	0.72	0.90	0.93	0.95	1.15	1.24	1.38	169	2004

(11) FT4	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	ng/dL										
Group GEL Children, Adolescents											
0 – 6 Days	0.69	0.86	0.97	1.34	1.38	1.45	2.29	2.50	2.69	111	2007
>6 Days ≤3 Months	0.76	0.89	0.97	1.33	1.35	1.40	2.08	2.20	2.34	113	2007
>3 ≤12 Months	0.83	0.92	0.97	1.32	1.34	1.36	1.91	1.99	2.09	117	2007
>1 ≤6 Years	0.91	0.96	0.98	1.30	1.31	1.32	1.72	1.77	1.83	344	2007
>6 ≤11 Years	0.94	0.97	0.99	1.28	1.29	1.31	1.63	1.67	1.72	263	2007
>11 ≤20 Years	0.95	0.98	0.99	1.27	1.28	1.29	1.59	1.63	1.67	469	2007

(12) FT4	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	ng/dL										
LIFE Child											
>3 ≤12 Months	0.98	1.04	1.07	1.23	1.26	1.30	1.49	1.59	1.63	83	2016
>1 ≤6 Years	1.00	1.04	1.06	1.23	1.26	1.28	1.52	1.56	1.60	193	2016
>6 ≤11 Years	0.97	1.00	1.02	1.22	1.24	1.26	1.50	1.53	1.58	243	2016
>11 ≤20 Years	0.87	0.90	0.93	1.16	1.19	1.21	1.50	1.53	1.62	206	2016

The different cohorts show that FT4 is quite stable over the life-time and that there are no big differences between male and female reference ranges.

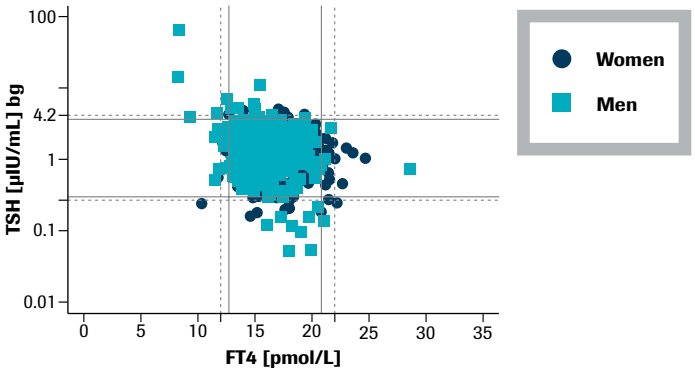
Over trimester 1 to 3, pregnant women show decreasing reference ranges. As also recommended in the guidelines from the American Thyroid Association, a trimester specific reference range for FT4 shall be applied.[12]

The group LIFE adult did not reveal any dependencies on BMI nor >30 neither >35 with a hip/waist ratio according the WHO. Furthermore, the reference ranges determined for healthy non-smokers, healthy former smokers and healthy smokers are comparable to each other.

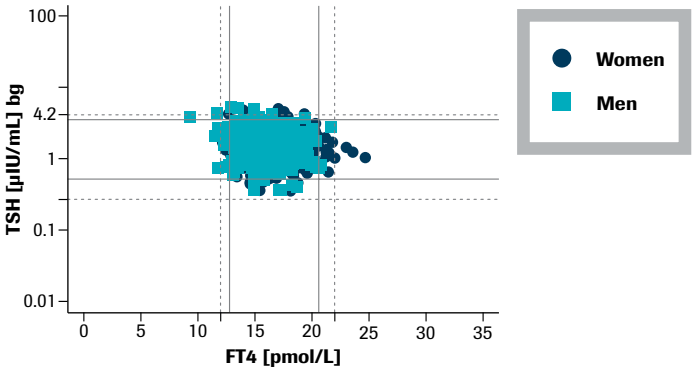
Correlation between Elecsys® TSH and Elecsys® FT4 results

In figure 8, each graph shows the Elecsys® FT4 results for the subjects of the individual groups GL1, GL2, GL3, pregnant women and LIFE adult, respectively, plotted against the Elecsys® TSH results of the same samples. Samples from male persons are indicated with rectangles and from female persons with circles. The dashed lines correspond to the 2.5 % and 97.5 % quantiles of the valid reference intervals as listed in the package inserts. The continuous lines represent the reference ranges determined in each group which is also stated in the legends. In these graphs, the dependency of the reference values on the applied inclusion/exclusion criteria as well as changes in the values during pregnancy becomes apparent. These results emphasize the importance of applying reference ranges adapted to specific cohorts e.g. trimester specific reference ranges for pregnant women.

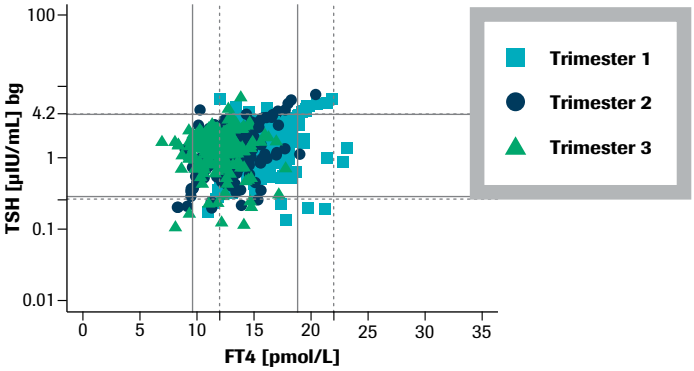
Group GL1 – all blood donners (n = 869 : 445 females, 424 males)



Group GL2 – TSH+Sono NAD (n = 643 : 338 females, 305 males)



Pregnant Women (n = 957 : 418 1st trim., 369 2nd trim., 170 3rd trim.)



Group GL3 (TSH NACB) (n = 447 : 274 females, 173 males)

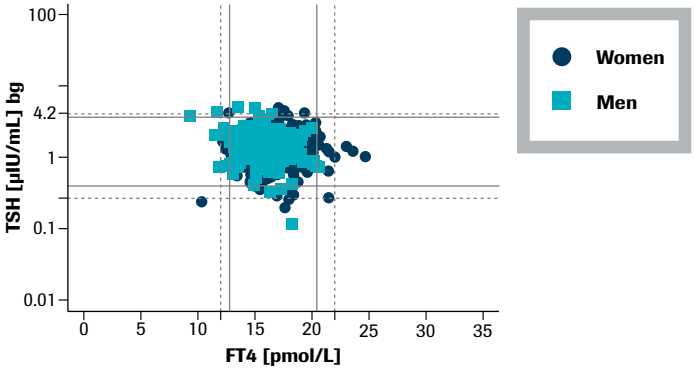
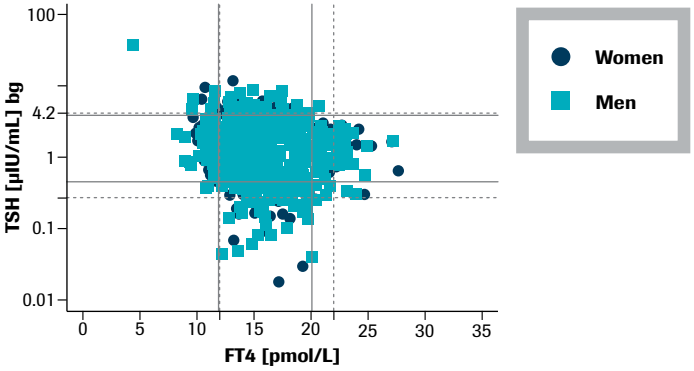


Figure 8: Correlation between Elecsys® FT4 and Elecsys® TSH results.

Group LIFE (healthy patients) (n = 5475 : 2,307 females, 3,168 males)



Reference ranges of Elecsys® FT4 under L-thyroxine supplementation

The figure 9 shows Elecsys® FT4 results of LIFE adult patients under thyroxine treatment filtered for euthyroid TSH (0.27 – 4.2 µIU/mL) and Anti-TPO negative patients. The results demonstrate a shift for Elecsys® FT4 measurements towards upper values. Evaluating the LIFE adult cohort shows a shift of reference ranges for patients under L-thyroxine supplementation to 13.61 – 28.29 pmol/L for Elecsys® FT4.

This finding emphasizes the importance of adapting reference ranges for patients under L-thyroxine supplementation treatment.

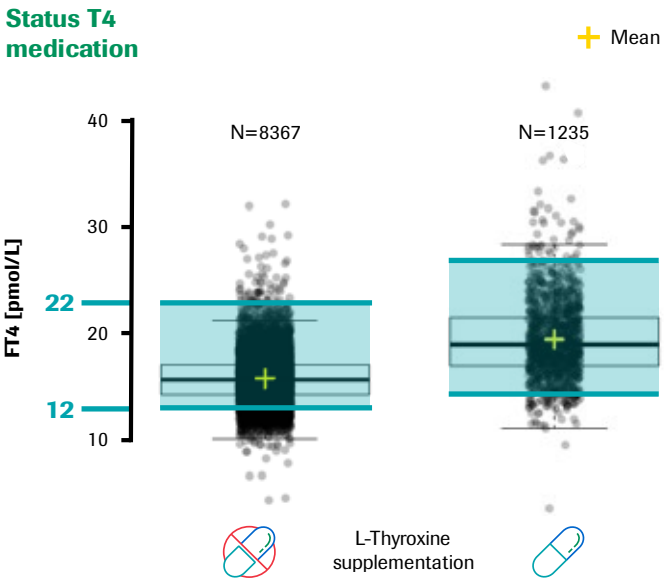


Figure 9: Filter – Distribution of FT4 [pmol/L] in patients under L-thyroxine supplementation and euthyroid TSH.

5 Results and Discussion

5.3 Free triiodothyronine – FT3

As for free T4, the determination of free T3 has the advantage of being independent of changes in the concentrations and binding properties of the binding proteins; additional determination of a binding parameter (T-uptake, TBG) is hence unnecessary. Therefore, free T3 is a useful tool in clinical routine diagnostics for the assessment of the thyroid status. Free T3 measurements support the differential diagnosis of thyroid disorders, are needed to distinguish different forms of hyperthyroidism, and to identify patients with T3 thyrotoxicosis.[13-15]

The tables 13 to 20 below describe the Elecsys® FT3 reference ranges determined in different studies for: 1. adults, separated into male and female and age, 2. pregnant women, separated into the 3 trimesters and 3. children, separated by age. All confidence intervals were computed with 95 % confidence level.

(13) FT3	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	pmol/L										
Elecsys® FT3 – package insert		3.1						6.8		5,366	2004
Group GL1 (all blood donors)	3.67	3.89	3.99	5.02	5.10	5.15	6.54	6.66	7.00	870	2004
L1 Males all	4.02	4.09	4.29	5.23	5.30	5.36	6.54	6.65	7.21	445	2004
L1 Males 20 – 39 y	4.04	4.36	4.54	5.22	5.29	5.37	6.54	6.74	7.21	286	2004
L1 Males 40 – 69 y	2.52	3.98	4.09	5.17	5.29	5.45	6.24	6.54	9.47	150	2004
L1 Females all	3.34	3.71	3.84	4.71	4.76	4.82	6.18	6.70	7.27	425	2004
L1 Females 20 – 39 y	3.13	3.66	3.89	4.72	4.78	4.88	6.10	6.72	7.94	270	2004
L1 Females 40 – 69 y	3.52	3.74	3.97	4.54	4.68	4.81	5.94	6.11	6.85	128	2004
L1 Females with contracept.	3.18	3.91	4.03	4.74	4.82	4.94	6.20	6.85	9.96	236	2004
L1 Females with contracept. 20 – 39 y	3.18	3.91	4.08	4.72	4.80	4.91	6.02	6.31	9.96	181	2004
L1 Females with contracept. 40 – 69 y	3.97	3.97	4.02	4.52	4.82	5.11	5.55	6.85	6.85	33	2004
L1 Females w/o contracept	3.01	3.55	3.74	4.61	4.70	4.80	6.10	6.37	7.94	188	2004
L1 Females w/o contracept. 20 – 39 y	3.01	3.34	3.8	4.63	4.74	4.91	5.67	6.87	7.94	88	2004
L1 Females w/o contracept. 40 – 69 y	3.52	3.71	3.92	4.47	4.63	4.77	5.94	6.11	6.37	95	2004
Group GL2 (TSH + SD-Sono NAD)	3.67	3.92	4.03	4.99	5.08	5.15	6.54	6.65	7.00	632	2004
L2 Males	3.85	4.07	4.28	5.21	5.25	5.35	6.54	6.66	7.21	332	2004
L2 Females	3.13	3.80	3.92	4.7	4.75	4.82	6.12	6.31	7.27	300	2004
Group GL3 org (NACB crit. for TSH)	3.71	3.92	4.07	5.03	5.12	5.19	6.54	6.74	7.21	448	2004
L3 Males	2.52	4.09	4.28	5.19	5.25	5.36	6.57	6.79	8.46	274	2004
L3 Females	3.01	3.80	3.89	4.70	4.77	4.85	6.10	6.70	9.96	174	2004
LIFE Adults	3.90	3.95	3.98	5.01	5.03	5.05	6.26	6.31	6.36	5,478	2013
all male patients (sd healthy)	4.08	4.14	4.19	5.14	5.17	5.20	6.35	6.41	6.50	3,170	2013
male <40 years	4.19	4.50	4.67	5.41	5.51	5.57	6.49	6.86	7.88	156	2013
male ≥40 and <70 years	4.15	4.23	4.29	5.20	5.23	5.26	6.38	6.44	6.53	2,324	2013
male ≥70 years	3.83	3.96	4.04	4.85	4.92	4.97	5.81	5.92	6.10	690	2013
all female patients (sd healthy)	3.77	3.84	3.89	4.81	4.84	4.87	5.94	6.03	6.12	2,308	2013
female <40 years	3.53	3.96	4.09	4.97	5.07	5.23	6.25	7.07	8.29	155	2013
female ≥40 and <70 years	3.79	3.86	3.90	4.83	4.85	4.89	5.94	6.02	6.13	1,755	2013
female ≥70 years	3.53	3.64	3.86	4.62	4.67	4.73	5.60	5.80	6.01	398	2013

(14) FT3	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	pmol/L										
Pregnant Women											
1st Trimester	3.60	3.78	3.87	4.74	4.80	4.87	5.84	5.97	6.22	416	2004
2nd Trimester	3.08	3.21	3.28	4.02	4.09	4.18	5.25	5.45	5.75	368	2004
3rd Trimester	2.44	3.09	3.19	3.80	3.90	3.99	4.73	5.03	6.36	169	2004

(15) FT3	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	pmol/L										
Group GEL Children, Adolescents											
0 – 6 Days	1.92	2.62	2.79	5.13	5.47	5.91	8.79	10.1	10.9	108	2007
>6 Days ≤3 Months	2.40	2.99	3.15	5.56	5.81	6.12	8.73	9.53	10.0	108	2007
>3 ≤12 Months	2.84	3.30	3.45	5.89	6.06	6.26	8.60	9.09	9.43	115	2007
>1 ≤6 Years	3.40	3.70	3.85	6.18	6.26	6.35	8.32	8.49	8.70	347	2007
>6 ≤11 Years	3.63	3.89	4.06	6.10	6.16	6.24	7.89	8.01	8.21	265	2007
>11 ≤20 Years	3.73	3.94	4.11	5.90	5.95	6.03	7.54	7.67	7.88	470	2007

(16) FT3	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	pmol/L										
LIFE Child											
>3 ≤12 Months	4.94	5.19	5.87	6.91	7.01	7.14	8.15	8.46	9.12	76	2016
>1 ≤6 Years	4.99	5.30	5.65	6.61	6.72	6.86	8.00	8.25	8.73	195	2016
>6 ≤11 Years	5.28	5.42	5.61	6.55	6.67	6.77	7.68	7.93	8.21	244	2016
>11 ≤20 Years	4.37	4.77	4.89	5.91	6.03	6.15	7.29	7.52	7.85	207	2016

(17) FT3	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	pg/mL										
Elecsys® FT3 – package insert		2.04						4.4		5,366	2004
Group GL1 (all blood donors)	2.39	2.53	2.60	3.27	3.32	3.35	4.26	4.34	4.56	870	2004
L1 Males all	2.62	2.66	2.79	3.40	3.45	3.49	4.26	4.33	4.69	445	2004
L1 Males 20 – 39 y	2.63	2.84	2.96	3.40	3.44	3.50	4.26	4.39	4.69	286	2004
L1 Males 40 – 69 y	1.64	2.59	2.66	3.37	3.45	3.55	4.06	4.26	6.16	150	2004
L1 Females all	2.17	2.42	2.50	3.07	3.10	3.14	4.02	4.36	4.73	425	2004
L1 Females 20 – 39 y	2.04	2.38	2.53	3.07	3.11	3.18	3.97	4.37	5.17	270	2004
L1 Females 40 – 69 y	2.29	2.43	2.58	2.96	3.05	3.13	3.87	3.98	4.46	128	2004
L1 Females with contracept.	2.07	2.55	2.62	3.09	3.14	3.22	4.04	4.46	6.48	236	2004
L1 Females with contracept. 20 – 39 y	2.07	2.55	2.66	3.07	3.12	3.20	3.92	4.11	6.48	181	2004
L1 Females with contracept. 40 – 69 y	2.58	2.58	2.62	2.94	3.14	3.33	3.61	4.46	4.46	33	2004
L1 Females w/o contracept	1.96	2.31	2.43	3.00	3.06	3.12	3.97	4.15	5.17	188	2004
L1 Females w/o contracept. 20 – 39 y	1.96	2.17	2.47	3.01	3.09	3.20	3.69	4.47	5.17	88	2004
L1 Females w/o contracept. 40 – 69 y	2.29	2.42	2.55	2.91	3.01	3.11	3.87	3.98	4.15	95	2004
Group GL2 (TSH + SD-Sono NAD)	2.39	2.55	2.62	3.25	3.31	3.35	4.26	4.33	4.56	632	2004
L2 Males	2.51	2.65	2.79	3.39	3.42	3.48	4.26	4.34	4.69	332	2004
L2 Females	2.04	2.47	2.55	3.06	3.10	3.14	3.98	4.11	4.73	300	2004
Group GL3 org (NACB crit. for TSH)	2.42	2.55	2.65	3.27	3.33	3.38	4.26	4.39	4.69	448	2004
L3 Males	1.64	2.66	2.79	3.38	3.42	3.49	4.28	4.42	5.51	274	2004
L3 Females	1.96	2.47	2.53	3.06	3.10	3.16	3.97	4.36	6.48	174	2004
LIFE Adults	2.54	2.57	2.59	3.26	3.27	3.29	4.08	4.11	4.14	5,478	2013
all male patients (sd healthy)	2.66	2.70	2.73	3.35	3.37	3.39	4.13	4.17	4.23	3,170	2013
male <40 years	2.73	2.93	3.04	3.52	3.59	3.63	4.22	4.47	5.13	156	2013
male ≥40 and <70 years	2.70	2.75	2.79	3.39	3.40	3.42	4.15	4.19	4.25	2,324	2013
male ≥70 years	2.49	2.58	2.63	3.16	3.20	3.24	3.78	3.85	3.97	690	2013
all female patients (sd healthy)	2.45	2.5	2.53	3.13	3.15	3.17	3.87	3.93	3.98	2,308	2013
female <40 years	2.30	2.58	2.66	3.24	3.30	3.40	4.07	4.60	5.40	155	2013
female ≥40 and <70 years	2.47	2.51	2.54	3.14	3.16	3.18	3.87	3.92	3.99	1,755	2013
female ≥70 years	2.30	2.37	2.51	3.01	3.04	3.08	3.65	3.78	3.91	398	2013

(18) FT3	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	pg/mL										
Pregnant Women											
1st Trimester	2.34	2.46	2.52	3.09	3.12	3.17	3.80	3.89	4.05	416	2004
2nd Trimester	2.01	2.09	2.14	2.62	2.66	2.72	3.42	3.55	3.74	368	2004
3rd Trimester	1.59	2.01	2.08	2.47	2.54	2.60	3.08	3.27	4.14	169	2004

(19) FT3	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	pg/mL										
Group GEL Children, Adolescents											
0 – 6 Days	1.25	1.71	1.81	3.34	3.56	3.85	5.72	6.58	7.12	108	2007
>6 Days ≤3 Months	1.57	1.95	2.05	3.62	3.78	3.98	5.69	6.20	6.54	108	2007
>3 ≤12 Months	1.85	2.15	2.24	3.83	3.95	4.07	5.60	5.92	6.14	115	2007
>1 ≤6 Years	2.21	2.41	2.51	4.02	4.08	4.13	5.41	5.53	5.67	347	2007
>6 ≤11 Years	2.37	2.53	2.64	3.97	4.01	4.07	5.13	5.21	5.34	265	2007
>11 ≤20 Years	2.43	2.57	2.68	3.84	3.88	3.92	4.91	4.99	5.13	470	2007

(20) FT3	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	pg/mL										
LIFE Child											
>3 ≤12 Months	3.21	3.38	3.82	4.50	4.56	4.65	5.30	5.51	5.93	76	2016
>1 ≤6 Years	3.25	3.45	3.68	4.30	4.38	4.47	5.21	5.37	5.68	195	2016
>6 ≤11 Years	3.44	3.53	3.65	4.27	4.34	4.4	5.00	5.16	5.34	244	2016
>11 ≤20 Years	2.85	3.10	3.18	3.85	3.93	4.01	4.74	4.89	5.11	207	2016

Establishing values reference ranges for the package insert is based primarily obtained on samples from one commercial laboratory. TSH and FT4 levels are found to be in the euthyroid range in these samples. The patients often have non-thyroid diseases which might influence the thyroid function in general, and especially the FT3 level (please see also chapter 6). This may explain the differences observed when comparing the reference ranges based on different population groups using the same Elecsys® FT3 method. Beside local differences in iodine intake the overall health status of the individuals involved is decisive for the outcome of the reference intervals.

The FT3 reference ranges show an age dependency. A slight difference in gender is seen especially for the 2.5 % quantile and the median. Males show higher values as compared to women. Pregnant women have lower FT3 concentrations compared to healthy, non-pregnant women correlated with the gestational age. As also recommended in the guidelines from the American Thyroid Association, a trimester specific reference range for FT3 shall be applied.[12]

The group LIFE adult did not reveal any dependencies on BMI nor >30 neither >35 with a hip/waist ratio according the WHO. Furthermore, the reference ranges determined for healthy non-smoker, healthy former smoker and healthy smokers are comparable to each other.

Figure 10 shows all data for children and adults from birth to 70 years using separate symbols to distinguish between male and female. The FT3 concentration decreases continuously with age. This emphasizes the importance of age-specific reference ranges.

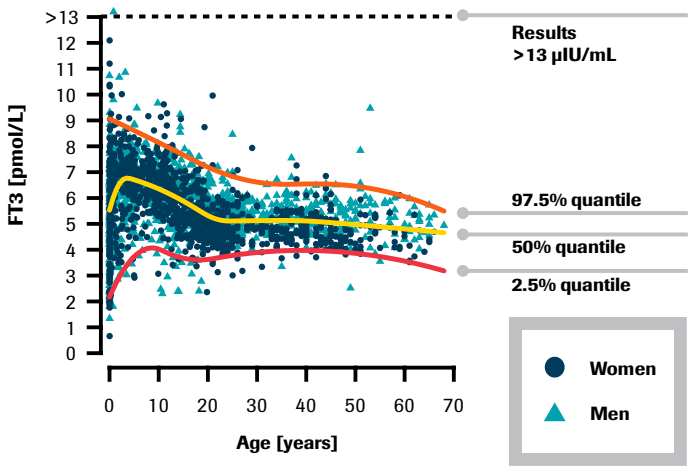
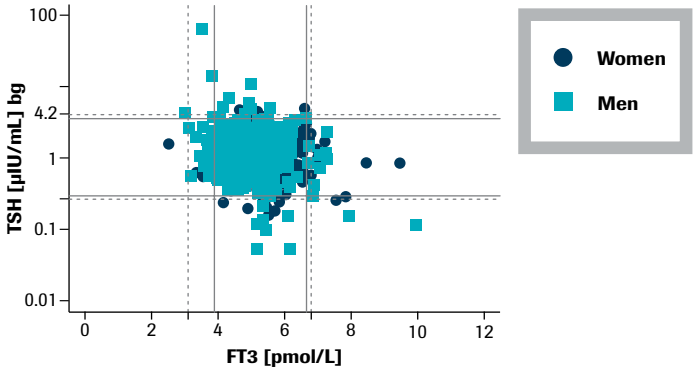


Figure 10: Quantile regression for Elecsys® FT3

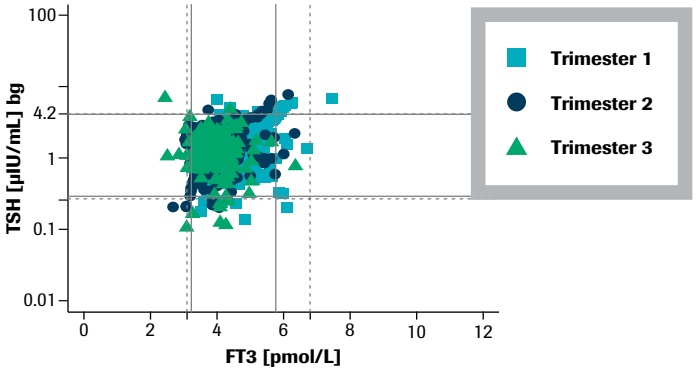
Correlation between Elecsys® TSH and Elecsys® FT3 results
In figure 11, each graph shows the Elecsys® FT3 results for the subjects of the individual groups GL1, GL2, GL3, pregnant women and LIFE adult, respectively, plotted against the Elecsys® TSH results of the same samples. Samples from male persons are indicated with rectangles and from female persons with circles. The dashed lines correspond to the 2.5 % and 97.5 % quantiles of the valid reference intervals as listed in the package inserts.

The continuous lines represent the reference ranges determined in each group which is also stated in the legends. In these graphs, the dependency of the reference values on the applied inclusion/exclusion criteria as well as changes in the values during pregnancy becomes apparent. These results emphasize the importance of applying reference ranges adapted to specific cohorts e.g. trimester specific reference ranges for pregnant women.

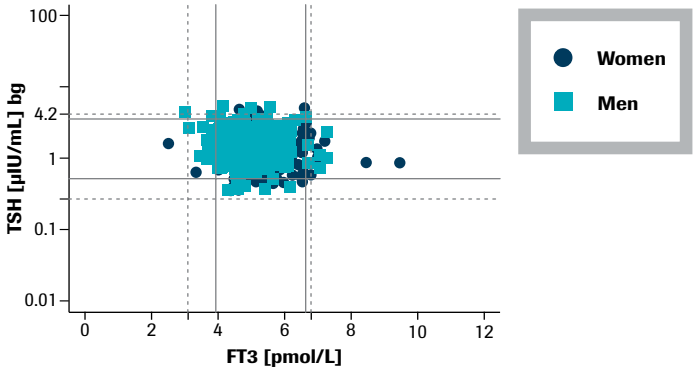
Group GL1 – all blood donners (n = 869 : 445 females, 424 males)



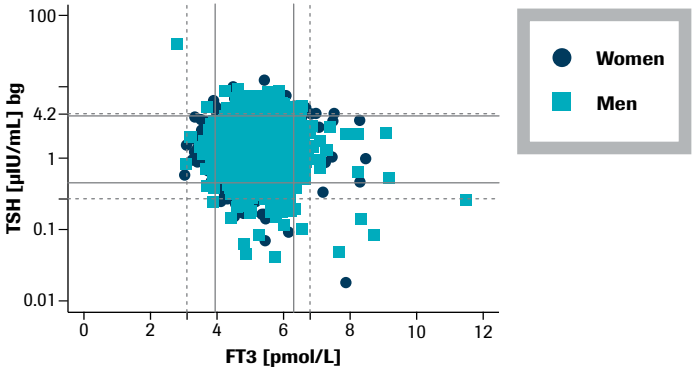
Pregnant Women (n = 957 : 418 1st trim., 369 2nd trim., 170 3rd trim.)



Group GL2 – TSH+Sono NAD (n = 643 : 338 females, 305 males)



Group LIFE (healthy patients) (n = 5476 : 2,308 females, 3,168 males)



Group GL3 (TSH NACB) (n = 447 : 274 females, 173 males)

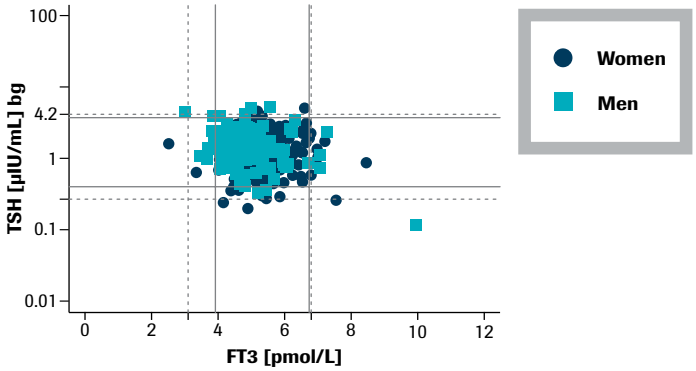


Figure 11: Correlation between Elecsys® FT3 and Elecsys® TSH results.

5 Results and Discussion

5.4 Thyroxine – T4

To measure thyroid hormones in serum, there are in principle two different approaches:

- Measurement of total T3 and T4 (TT3, TT4)
- Measurement of free T3 and T4 (FT3 ~0.3 % and FT4 ~0.03 % of the TT3 and TT4, respectively)

T4 in serum is bound to proteins in over 99.9 %. The major binding protein is the thyroxine-binding-globulin (TBG). In much lower frequency transthyretin (TTR) and in low amounts albumin bind thyroid hormones. T3 is also bound to TBG, but with an about 10-fold lower affinity than T4, to albumin and in very low frequency also to TTR.

Changes of the binding protein concentration or binding protein capacity leads to a change in the total hormone level while the free hormone level, and therefore the thyroid function, remains unchanged. This is the case e.g. in pregnancy, during treatment with certain medications and in patients with genetic abnormalities in the binding proteins.

If the total thyroid hormones are measured, it is recommended to determine the thyroxine-binding capacity to take into account influences caused by varying concentrations of binding proteins. The free thyroxine index can be calculated (e.g. measurements of TBG and T-uptake).[3,16]

The tables 21 to 26 below describe the Elecsys® T4 reference ranges determined in different studies for: 1. adults, separated into male and female and age, 2. pregnant women, separated into the 3 trimesters and 3. children, separated by age.

(21) T4	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
					nmol/L						
Elecsys® T4 – insert Europe/Japan		66						181		2,526	1998
Elecsys® T4 – insert USA, 99 % centr.		58						155		235	1998
Group GL1 (all blood donors)	68.3	71.5	72.7	99.4	101	103	152	158	163	870	2004
L1 Males all	65.8	71.6	72.7	93.5	95.9	97.3	125	128	130	445	2004
L1 Males 20 – 39 y	62.6	71.8	73.6	92.2	94.7	96.6	122	125	130	286	2004
L1 Males 40 – 69 y	52.1	68.4	75.4	95.3	97.4	100	124	129	144	150	2004
L1 Females all	63.5	71.5	75.6	108	110	114	161	166	192	425	2004
L1 Females 20 – 39 y	70.0	76.2	80.0	113	116	119	160	166	193	270	2004
L1 Females 40 – 69 y	55.2	63.5	72.5	95.9	99.7	105	143	157	168	128	2004
L1 Females with contracept.	70.0	81.8	89.4	121	124	126	166	173	209	236	2004
L1 Females with contracept. 20 – 39 y	70.0	84.5	91.2	120	123	126	162	172	198	181	2004
L1 Females with contracept. 40 – 69 y	75.8	75.8	83.7	105	118	135	163	168	168	33	2004
L1 Females w/o contracept	55.2	68.0	71.5	92.9	95.7	99.0	126	138	170	188	2004
L1 Females w/o contracept. 20 – 39 y	69.1	71.5	76.5	91.7	96.1	103	122	138	161	88	2004
L1 Females w/o contracept. 40 – 69 y	55.2	59.8	70.5	92.1	95.7	101	120	127	139	95	2004
Group GL2 (TSH + SD-Sono NAD)	68.3	71.4	73.1	98.4	100	103	152	160	166	632	2004
L2 Males	62.6	68.9	72.7	92.1	94.6	96.4	122	124	131	332	2004
L2 Females	68.0	71.5	76.5	109	113	116	161	166	175	300	2004
Group GL3 org (NACB crit. for TSH)	68.0	69.1	71.8	96.1	98.5	101	150	159	166	448	2004
L3 Males	61.8	68.4	71.8	90.6	93.0	96.3	122	125	144	274	2004
L3 Females	63.5	71.4	76.5	107	113	118	161	166	173	174	2004

(22) T4	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
					nmol/L						
Pregnant Women											
1st Trimester	87.9	94.4	96.4	126	130	132	186	191	210	417	2004
2nd Trimester	96.7	102	106	144	147	151	197	208	228	368	2004
3rd Trimester	81.4	89.5	104	140	145	149	193	202	279	169	2004

(23) T4	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
					nmol/L						
Group GEL Children, Adolescents											
0 – 6 Days	52.9	64.8	74.9	138	148	156	227	240	284	92	2007
>6 Days ≤3 Months	60.3	69.6	75.9	132	138	142	211	219	247	101	2007
>3 ≤12 Months	65.2	72.9	78.0	128	131	134	199	206	223	105	2007
>1 ≤6 Years	70.9	76.5	80.0	120	122	124	181	189	196	341	2007
>6 ≤11 Years	72.8	77.0	80.0	114	116	118	170	177	181	264	2007
>11 ≤20 Years	72.8	76.1	78.4	111	112	114	164	170	174	470	2007

(24) T4	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
					µg/dL						
Elecsys® T4 – insert Europe/Japan		5.1						14.1		2,526	1998
Elecsys® T4 – insert USA, 99 % centr.		4.5						12.1		235	1998
Group GL1 (all blood donors)	5.31	5.56	5.65	7.72	7.84	7.99	11.8	12.2	12.7	870	2004
L1 Males all	5.11	5.56	5.65	7.26	7.45	7.56	9.67	9.91	10.1	445	2004
L1 Males 20 – 39 y	4.86	5.57	5.72	7.16	7.36	7.51	9.51	9.69	10.1	286	2004
L1 Males 40 – 69 y	4.05	5.32	5.86	7.40	7.57	7.80	9.59	13.0	11.2	150	2004
L1 Females all	4.93	5.56	5.88	8.38	8.58	8.86	12.5	12.9	14.9	425	2004
L1 Females 20 – 39 y	5.44	5.92	6.22	8.79	9.01	9.22	12.4	12.9	15.0	270	2004
L1 Females 40 – 69 y	4.29	4.93	5.63	7.45	7.75	8.17	11.1	12.2	13.0	128	2004
L1 Females with contracept.	5.44	6.35	6.94	9.37	9.61	9.82	12.9	13.4	16.3	236	2004
L1 Females with contracept. 20 – 39 y	5.44	6.64	7.09	9.34	9.57	9.82	12.6	13.3	15.4	181	2004
L1 Females with contracept. 40 – 69 y	5.88	5.88	6.50	8.17	9.14	10.5	12.6	13.0	13.0	33	2004
L1 Females w/o contracept	4.29	5.29	5.56	7.21	7.43	7.69	9.80	10.7	13.2	188	2004
L1 Females w/o contracept. 20 – 39 y	5.37	5.56	5.95	7.12	7.46	7.96	9.45	10.7	12.5	88	2004
L1 Females w/o contracept. 40 – 69 y	4.29	4.64	5.48	7.16	7.44	7.85	9.36	9.90	10.8	95	2004
Group GL2 (TSH + SD-Sono NAD)	5.31	5.55	5.68	7.64	7.81	8.01	11.8	12.4	12.9	632	2004
L2 Males	4.86	5.35	5.65	7.15	7.35	7.49	9.51	9.64	10.2	332	2004
L2 Females	5.29	5.56	5.95	8.48	8.79	9.02	12.5	12.9	13.6	300	2004
Group GL3 org (NACB crit. for TSH)	5.29	5.37	5.58	7.47	7.65	7.84	11.7	12.3	12.9	448	2004
L3 Males	4.80	5.31	5.58	7.04	7.23	7.48	9.51	9.71	11.2	274	2004
L3 Females	4.93	5.55	5.95	8.32	8.80	9.19	12.5	12.9	13.4	174	2004

(25) T4	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
					µg/dL						
Pregnant Women											
1st Trimester	6.83	7.33	7.49	9.80	10.1	10.3	14.4	14.8	16.3	417	2004
2nd Trimester	7.51	7.93	8.23	11.2	11.4	11.8	15.3	16.1	17.7	368	2004
3rd Trimester	6.33	6.95	8.06	10.9	11.3	11.6	15.0	15.7	21.7	169	2004

(26) T4	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
					µg/dL						
Group GEL Children, Adolescents											
0 – 6 Days	4.11	5.04	5.82	10.7	11.5	12.1	17.6	18.6	22.1	92	2007
>6 Days ≤3 Months	4.69	5.41	5.90	10.2	10.7	11.0	16.4	17.0	19.2	101	2007
>3 ≤12 Months	5.06	5.67	6.06	9.91	10.2	10.4	15.5	16.0	17.3	105	2007
>1 ≤6 Years	5.51	5.95	6.21	9.35	9.50	9.61	14.1	14.7	15.3	341	2007
>6 ≤11 Years	5.66	5.99	6.21	8.89	9.03	9.14	13.2	13.8	14.1	264	2007
>11 ≤20 Years	5.66	5.91	6.09	8.59	8.73	8.83	12.8	13.2	13.5	470	2007

The data of the different cohorts basically confirm the reference range stated in the package insert. The reference limits are a bit narrower for Elecsys® T4 which reflects local differences and the influence of the inclusion/exclusion criteria for the selection of the cohort. The data in the group from Austria (Group A) correspond to those of the group of blood donors.
A clear dependency on the increased TBG concentration caused by the intake of contraceptives could be observed. Significantly higher Elecsys® T4 results were seen in the group of women taking contraceptives (GL1 females contraceptives) compared to the group of women not taking contraceptives (GL1 females without contraceptives). The results from the group of women not taking contraceptives approximate to those of the group GL1 males. Pregnant women have higher Elecsys® T4 concentrations correlated with the gestational age.

Correlation between Elecsys® TSH and Elecsys® T4 results
In figure 12, each graph shows the Elecsys® T4 results for the subjects of the individual groups GL1, GL2, GL3 and pregnant women, respectively, plotted against the Elecsys® TSH results of the same samples. Samples from male persons are indicated with rectangles and from female persons with circles. The dashed lines correspond to the 2.5 % and 97.5 % quantiles of the valid reference intervals as listed in the package inserts. The continuous lines represent the reference ranges determined in each group which is also stated in the legends. In these graphs, the dependency of the reference values on the applied inclusion/exclusion criteria as well as changes in the values during pregnancy becomes apparent. These results emphasize the importance of applying reference ranges adapted to specific cohorts e.g. trimester specific reference ranges for pregnant women.

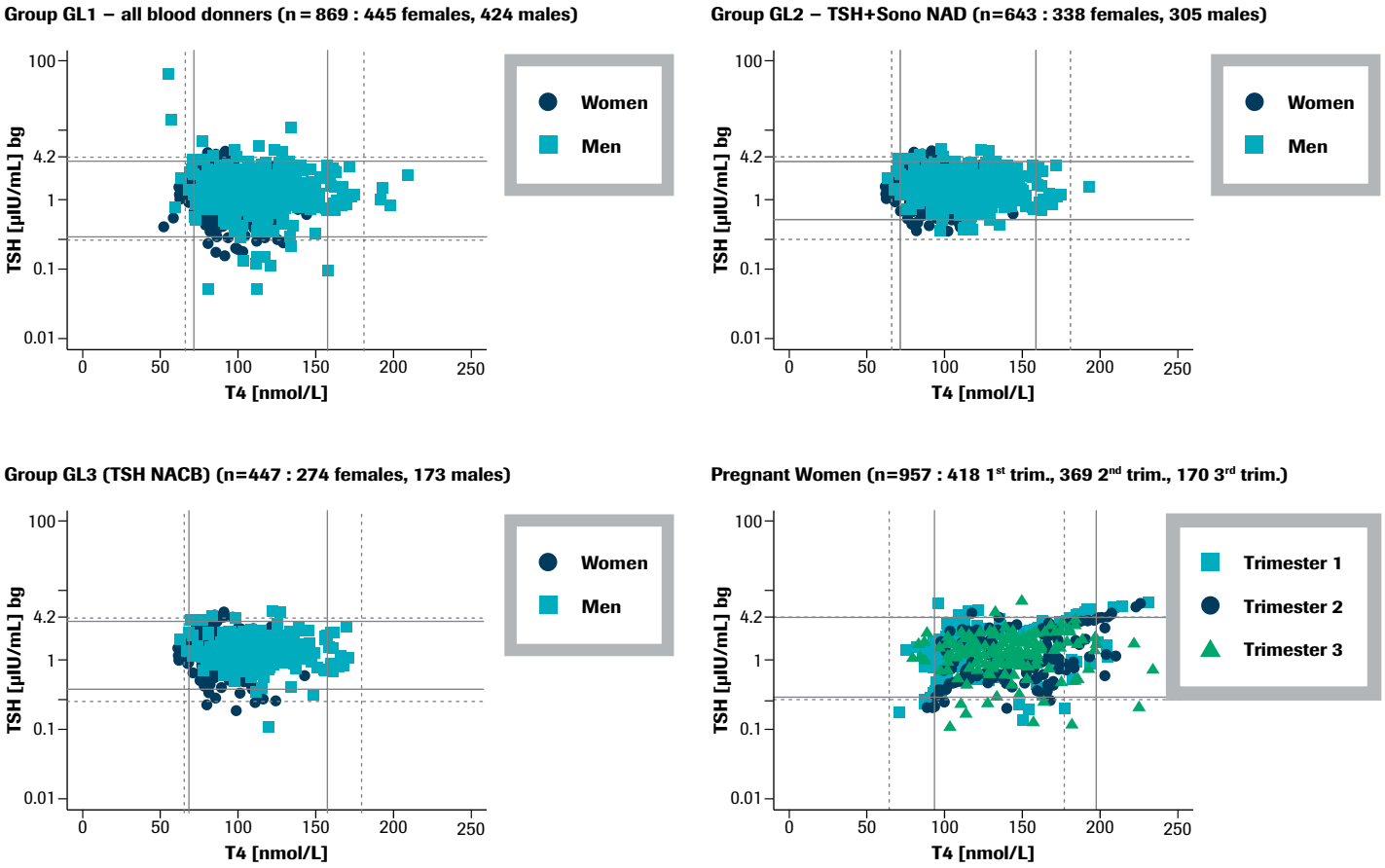


Figure 12: Correlation between Elecsys® T4 and Elecsys® TSH results.

5 Results and Discussion

5.5 Thyroxine-binding capacity – Free T4-Index – FT4I (T4/TBI)

As the major part of the total thyroxine is bound to transport proteins (TBG, prealbumin, and albumin), the determination of total thyroxine only provides correct information when the thyroxine-binding capacity in serum is normal. The free thyroid hormones are in equilibrium with the hormones bound to the carrier proteins. A change in the TBG concentration can lead to elevated or lowered total T4 concentrations being measured although the Free T4 concentration is in the euthyroid range.

The performance of a T-uptake or TBC assay provides a measure of the available thyroxine-binding sites. Determination of the free thyroxine index (FT4I) from the ratio of total T4 and TBI (thyroxine-binding index = result of the T-uptake determination) takes into account changes in the thyroid hormone carrier proteins and the thyroxine level.

The tables 27 to 29 below describe the Elecsys® T-uptake and the FT4I reference ranges determined in different studies for: 1. adults, separated into male and female and age, 2. pregnant women, separated into the 3 trimesters and 3. children, separated by age.

(27) T-uptake	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	TBI										
Elecsys® T-uptake – insert Europe/ Japan		0.8						1.30		974	1998
Group GL1 (all blood donors)	0.80	0.81	0.84	0.99	0.99	1.00	1.22	1.23	1.25	870	2004
L1 Males all	0.77	0.80	0.82	0.96	0.96	0.97	1.06	1.08	1.09	445	2004
L1 Males 20 – 39 y	0.77	0.80	0.83	0.96	0.97	0.97	1.05	1.07	1.09	286	2004
L1 Males 40 – 69 y	0.70	0.77	0.85	0.95	0.97	0.99	1.07	1.09	1.18	150	2004
L1 Females all	0.75	0.85	0.87	1.04	1.05	1.07	1.25	1.26	1.30	425	2004
L1 Females 20 – 39 y	0.75	0.84	0.90	1.06	1.08	1.11	1.25	1.27	1.32	270	2004
L1 Females 40 – 69 y	0.56	0.84	0.87	0.99	1.01	1.03	1.19	1.22	1.31	128	2004
L1 Females with contracept.	0.80	0.94	0.96	1.11	1.12	1.13	1.26	1.27	1.32	236	2004
L1 Females with contracept. 20 – 39 y	0.80	0.94	0.96	1.11	1.12	1.14	1.26	1.27	1.32	181	2004
L1 Females with contracept. 40 – 69 y	0.95	0.95	1.00	1.07	1.09	1.13	1.25	1.31	1.31	33	2004
L1 Females w/o contracept	0.56	0.81	0.84	0.98	0.99	1.00	1.13	1.16	1.34	188	2004
L1 Females w/o contracept. 20 – 39 y	0.73	0.81	0.85	0.98	0.99	1.00	1.12	1.16	1.34	88	2004
L1 Females w/o contracept. 40 – 69 y	0.56	0.81	0.86	0.96	0.98	1.00	1.11	1.16	1.20	95	2004
Group GL2 (TSH + SD-Sono NAD)	0.77	0.81	0.84	0.99	1.00	1.01	1.23	1.25	1.27	632	2004
L2 Males	0.76	0.80	0.82	0.96	0.97	0.98	1.06	1.08	1.09	332	2004
L2 Females	0.73	0.86	0.90	1.05	1.07	1.09	1.25	1.27	1.32	300	2004
Group GL3 org (NACB crit. for TSH)	0.77	0.81	0.83	0.98	0.99	1.00	1.22	1.25	1.27	448	2004
L3 Males	0.70	0.80	0.82	0.96	0.97	0.98	1.07	1.09	1.18	274	2004
L3 Females	0.73	0.84	0.90	1.05	1.08	1.10	1.25	1.27	1.34	174	2004

(28) T-uptake	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	TBI										
Pregnant Women											
1st Trimester	0.91	0.96	0.98	1.10	1.11	1.12	1.29	1.31	1.35	415	2004
2nd Trimester	1.06	1.17	1.18	1.32	1.33	1.34	1.46	1.48	1.51	369	2004
3rd Trimester	0.84	1.28	1.28	1.39	1.41	1.42	1.50	1.52	1.55	169	2004

29 T-uptake	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	TBI										
Group GEL Children, Adolescents											
0 – 6 Days	0.72	0.81	0.85	1.01	1.02	1.05	1.18	1.20	1.25	61	2007
>6 Days ≤3 Months	0.74	0.81	0.83	1.00	1.01	1.03	1.19	1.21	1.23	64	2007
>3 ≤12 Months	0.76	0.80	0.83	1.00	1.00	1.01	1.19	1.21	1.23	88	2007
>1 ≤6 Years	0.77	0.80	0.83	0.99	1.00	1.00	1.20	1.22	1.24	337	2007
>6 ≤11 Years	0.78	0.80	0.83	0.99	0.99	1.00	1.20	1.22	1.24	254	2007
>11 ≤20 Years	0.78	0.80	0.82	0.99	0.99	1.00	1.21	1.22	1.24	462	2007

The data provided from the different groups basically confirm the reference interval currently given in the Elecsys® T-Uptake package insert.

Looking at the group of women with and without taking contraceptives, a dependency on the increased TBG concentration caused by the intake of contraceptives could be observed for T-uptake.

The median value of 1.0 TBI which was set arbitrarily for a group of healthy subjects (women without taking contraceptives) was confirmed in all groups evaluated.

As expected T-uptake concentrations are increasing with gestational age. The median values in the 1st, 2nd and 3rd trimester differ highly significant ($p<0.05$) between each other.

The table 30 to 35 below describe the FT4I determined from total T4 and TBI (thyroxine-binding index = result of the T-uptake determination) in different studies for 1. adults, separated into male and female and age, 2. pregnant women, separated into the 3 trimester and 3. children, separated by age.

The data provided from the different groups basically confirm the reference intervals calculated for the FT4-index from T4 and TBI (T4/TBI) currently given in the Elecsys® T-Uptake package insert.

Looking at the group of women with and without taking contraceptives, a dependency on the increased TBG concentration caused by the intake of contraceptives could also be observed for FT4I.

As expected FT4-index concentrations are decreasing with gestational age. The median values in the 1st, 2nd and 3rd trimester show highly significant ($p<0.05$) differences.

(30) FT4I (T4/TBI)	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	nmol/L										
FT4 Index – Insert Europe/Japan		62.0						164		825	1998
FT4 Index – Insert USA, 99 % centr.		55.0						139		233	1998
Group GL1 (all blood donors)	68.6	72.2	74.4	101	102	103	145	147	154	870	2004
L1 Males all	67.6	72.2	74.4	97.7	99.4	101	135	138	152	445	2004
L1 Males 20 – 39 y	67.1	73.0	75.8	97.8	98.7	101	132	136	152	286	2004
L1 Males 40 – 69 y	58.5	68.7	76.1	96.7	101	104	135	145	153	150	2004
L1 Females all	66.0	72.9	76.3	103	105	107	147	154	176	425	2004
L1 Females 20 – 39 y	70.7	76.4	80.1	103	106	108	146	154	191	270	2004
L1 Females 40 – 69 y	48.0	66.0	74.3	97.5	101	106	131	142	165	128	2004
L1 Females with contracept.	70.8	79.7	82.9	108	109	112	148	156	204	236	2004
L1 Females with contracept. 20 – 39 y	74.6	81.8	83.4	106	109	112	146	154	204	181	2004
L1 Females with contracept. 40 – 69 y	70.8	70.8	79.7	95.1	108	121	137	148	148	33	2004
L1 Females w/o contracept	48.0	66.1	72.9	96.0	98.7	102	134	148	173	188	2004
L1 Females w/o contracept. 20 – 39 y	70.1	72.9	76.8	93.8	98.4	103	125	148	173	88	2004
L1 Females w/o contracept. 40 – 69 y	48.0	60.8	70.9	94.0	98.9	105	125	142	165	95	2004
Group GL2 (TSH + SD-Sono NAD)	68.7	72.2	74.6	99.6	102	103	143	147	154	632	2004
L2 Males	67.1	71.8	73.8	96.5	98.1	101	134	137	153	332	2004
L2 Females	66.0	74.4	76.9	103	105	107	146	150	176	300	2004
Group GL3 org (NACB crit. for TSH)	68.0	71.8	73.5	97.7	100	102	137	147	154	448	2004
L3 Males	64.0	70.3	73.0	95.5	97.5	100	133	137	161	274	2004
L3 Females	60.8	72.9	76.9	100	103	107	146	153	176	174	2004

(31) FT4I (T4/TBI)	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	nmol/L										
Pregnant Women											
1st Trimester	79.6	83.2	85.9	113	116	118	158	166	176	414	2004
2nd Trimester	71.7	75.8	78.5	109	112	115	153	159	184	368	2004
3rd Trimester	55.4	65.8	69.6	101	103	107	142	160	199	168	2004

(32) FT4I (T4/TBI)	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	nmol/L										
Group GEL Children, Adolescents											
0 – 6 Days	48.1	65.3	86.4	124	130	140	219	253	278	47	2007
>6 Days ≤3 Months	59.3	72.4	85.8	122	126	131	204	218	235	47	2007
>3 ≤12 Months	66.3	76.0	85.5	121	124	127	194	202	217	76	2007
>1 ≤6 Years	75.8	80.2	84.1	118	120	121	178	182	194	283	2007
>6 ≤11 Years	77.5	80.6	83.0	115	116	117	166	169	180	150	2007
>11 ≤20 Years	76.9	78.8	80.6	111	112	113	159	162	171	306	2007

33 FT4I (T4/TBI)	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	µg/dL										
FT4 Index – Insert Europe/Japan		4.8						12.7		825	1998
FT4 Index – USA, 99 % centr.		4.2						10.8		233	1998
Group GL1 (all blood donors)	5.33	5.61	5.78	7.85	7.94	8.03	11.2	11.5	12.0	870	2004
L1 Males all	5.25	5.61	5.78	7.59	7.72	7.87	10.5	10.7	11.8	445	2004
L1 Males 20 – 39 y	5.21	5.67	5.89	7.52	7.67	7.86	10.3	10.5	11.8	286	2004
L1 Males 40 – 69 y	4.54	5.34	5.91	7.51	7.83	8.08	10.5	11.2	11.9	150	2004
L1 Females all	5.13	5.66	5.93	8.00	8.17	8.30	11.4	12.0	13.7	425	2004
L1 Females 20 – 39 y	5.49	5.94	6.22	8.03	8.20	8.41	11.4	12.0	14.9	270	2004
L1 Females 40 – 69 y	3.73	5.13	5.77	7.58	7.85	8.25	10.2	11.0	12.8	128	2004
L1 Females with contracept.	5.50	6.19	6.44	8.35	8.46	8.70	11.5	12.1	15.8	236	2004
L1 Females with contracept. 20 – 39 y	5.79	6.36	6.48	8.26	8.46	8.72	11.4	12.0	15.8	181	2004
L1 Females with contracept. 40 – 69 y	5.50	5.50	6.19	7.39	8.41	9.37	10.7	11.5	11.5	33	2004
L1 Females w/o contracept	3.73	5.13	5.66	7.46	7.67	7.91	10.4	11.5	13.5	188	2004
L1 Females w/o contracept. 20 – 39 y	5.45	5.66	5.96	7.28	7.64	8.00	9.73	11.5	13.5	88	2004
L1 Females w/o contracept. 40 – 69 y	3.73	4.72	5.51	7.30	7.68	8.16	9.67	11.0	12.8	95	2004
Group GL2 (TSH + SD-Sono NAD)	5.34	5.61	5.79	7.74	7.89	7.99	11.1	11.4	12.0	632	2004
L2 Males	5.21	5.58	5.74	7.49	7.62	7.84	10.4	10.7	11.9	332	2004
L2 Females	5.13	5.78	5.98	7.96	8.11	8.29	11.3	11.6	13.7	300	2004
Group GL3 org (NACB crit. for TSH)	5.25	5.58	5.71	7.59	7.74	7.94	10.7	11.4	12.0	448	2004
L3 Males	4.98	5.46	5.67	7.42	7.57	7.77	10.4	10.7	12.5	274	2004
L3 Females	4.72	5.66	5.98	7.79	8.01	8.29	11.4	11.9	13.7	174	2004

34 FT4I (T4/TBI)	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	µg/dL										
Pregnant Women											
1st Trimester	6.18	6.46	6.68	8.78	9.02	9.20	12.3	12.9	13.7	414	2004
2nd Trimester	5.57	5.89	6.10	8.48	8.71	8.91	11.9	12.4	14.3	368	2004
3rd Trimester	4.30	5.11	5.41	7.82	7.98	8.31	11.0	12.4	15.5	168	2004

35 FT4I (T4/TBI)	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	µg/dL										
Group GEL Children, Adolescents											
0 – 6 Days	3.74	5.08	6.71	9.61	10.1	10.9	17.0	19.6	21.6	47	2007
>6 Days ≤3 Months	4.61	5.63	6.66	9.49	9.77	10.2	15.8	16.9	18.2	47	2007
>3 ≤12 Months	5.15	5.90	6.64	9.43	9.60	9.87	15.1	15.7	16.9	76	2007
>1 ≤6 Years	5.89	6.23	6.54	9.20	9.30	9.43	13.8	14.1	15.1	283	2007
>6 ≤11 Years	6.02	6.26	6.45	8.90	9.00	9.10	12.9	13.2	14.0	150	2007
>11 ≤20 Years	5.97	6.13	6.26	8.63	8.72	8.81	12.3	12.6	13.3	306	2007

5 Results and Discussion

5.6 Triiodothyronine – T3

Please see also 5.4 Thyroxine – T4

The tables 36 to 41 below describe the Elecsys® T3 reference ranges determined in different studies for: 1. adults, separated into male and female and age, 2. pregnant women, separated into the 3 trimesters and 3. children, separated by age.

36 T3	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
					nmol/L						
Elecsys® T3 – package insert		1.3						3.1		514	1996
Group GL1 (all blood donors)	1.17	1.23	1.28	1.75	1.77	1.81	2.70	2.80	2.90	870	2004
L1 Males all	1.17	1.28	1.31	1.68	1.72	1.75	2.24	2.29	2.47	445	2004
L1 Males 20 – 39 y	1.24	1.30	1.35	1.65	1.69	1.74	2.20	2.29	2.34	286	2004
L1 Males 40 – 69 y	0.90	1.17	1.31	1.70	1.73	1.81	2.20	2.42	3.36	150	2004
L1 Females all	1.11	1.20	1.26	1.82	1.88	1.94	2.84	2.92	3.44	425	2004
L1 Females 20 – 39 y	1.08	1.20	1.28	1.90	1.97	2.03	2.81	2.90	3.50	270	2004
L1 Females 40 – 69 y	0.98	1.17	1.28	1.58	1.64	1.76	2.37	2.70	3.26	128	2004
L1 Females with contracept.	1.20	1.33	1.48	2.06	2.12	2.19	2.89	3.26	4.00	236	2004
L1 Females with contracept. 20 – 39 y	1.20	1.33	1.48	2.06	2.14	2.20	2.81	2.92	3.96	181	2004
L1 Females with contracept. 40 – 69 y	1.42	1.42	1.55	1.84	1.99	2.21	2.70	3.26	3.26	33	2004
L1 Females w/o contracept	0.98	1.13	1.19	1.58	1.62	1.66	2.25	2.79	2.92	188	2004
L1 Females w/o contracept. 20 – 39 y	1.06	1.11	1.24	1.59	1.66	1.73	2.12	2.84	2.92	88	2004
L1 Females w/o contracept. 40 – 69 y	0.98	1.14	1.23	1.50	1.57	1.64	2.20	2.40	2.77	95	2004
Group GL2 (TSH + SD-Sono NAD)	1.13	1.24	1.28	1.75	1.79	1.83	2.70	2.81	2.99	632	2004
L2 Males	1.13	1.28	1.31	1.66	1.72	1.75	2.23	2.29	2.51	332	2004
L2 Females	1.08	1.23	1.28	1.85	1.92	1.99	2.88	2.97	3.50	300	2004
Group GL3 org (NACB crit. for TSH)	1.14	1.26	1.30	1.73	1.77	1.81	2.66	2.75	2.97	448	2004
L3 Males	0.89	1.28	1.31	1.65	1.70	1.75	2.21	2.29	2.66	274	2004
L3 Females	1.06	1.23	1.30	1.85	1.94	2.01	2.88	2.97	3.96	174	2004

37 T3	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
					nmol/L						
Pregnant Women											
1st Trimester	1.52	1.61	1.66	2.18	2.24	2.28	3.28	3.53	3.91	415	2004
2nd Trimester	1.83	1.98	2.15	2.77	2.84	2.91	3.79	4.03	4.40	368	2004
3rd Trimester	1.87	2.08	2.21	3.00	3.10	3.18	3.93	4.02	5.01	169	2004

38 T3	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
					nmol/L						
Group GEL Children, Adolescents											
0 – 6 Days	0.88	1.11	1.21	2.31	2.45	2.75	4.20	4.80	4.99	100	2007
>6 Days ≤3 Months	1.03	1.23	1.31	2.45	2.54	2.73	4.09	4.43	4.58	96	2007
>3 ≤12 Months	1.18	1.32	1.39	2.54	2.60	2.71	3.96	4.18	4.33	101	2007
>1 ≤6 Years	1.33	1.42	1.48	2.57	2.61	2.66	3.68	3.81	3.98	342	2007
>6 ≤11 Years	1.36	1.43	1.49	2.44	2.49	2.53	3.41	3.52	3.70	267	2007
>11 ≤20 Years	1.35	1.40	1.45	2.30	2.34	2.38	3.22	3.32	3.48	473	2007

(39) T3	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	ng/mL										
Elecsys® T3 – package insert		0.8						2.0		514	1996
Group GL1 (all blood donors)	0.76	0.8	0.83	1.14	1.15	1.18	1.76	1.82	1.89	870	2004
L1 Males all	0.76	0.83	0.85	1.09	1.12	1.14	1.46	1.49	1.61	445	2004
L1 Males 20 – 39 y	0.81	0.85	0.88	1.07	1.10	1.13	1.43	1.49	1.52	286	2004
L1 Males 40 – 69 y	0.59	0.76	0.85	1.11	1.13	1.18	1.43	1.58	2.19	150	2004
L1 Females all	0.72	0.78	0.82	1.18	1.22	1.26	1.85	1.90	2.24	425	2004
L1 Females 20 – 39 y	0.70	0.78	0.83	1.24	1.28	1.32	1.83	1.89	2.28	270	2004
L1 Females 40 – 69 y	0.64	0.76	0.83	1.03	1.07	1.15	1.54	1.76	2.12	128	2004
L1 Females with contracept.	0.78	0.87	0.96	1.34	1.38	1.43	1.88	2.12	2.60	236	2004
L1 Females with contracept. 20 – 39 y	0.78	0.87	0.96	1.34	1.39	1.43	1.83	1.90	2.58	181	2004
L1 Females with contracept. 40 – 69 y	0.92	0.92	1.01	1.20	1.30	1.44	1.76	2.12	2.12	33	2004
L1 Females w/o contracept	0.64	0.74	0.77	1.03	1.05	1.08	1.46	1.82	1.90	188	2004
L1 Females w/o contracept. 20 – 39 y	0.69	0.72	0.81	1.04	1.08	1.13	1.38	1.85	1.90	88	2004
L1 Females w/o contracept. 40 – 69 y	0.64	0.74	0.80	0.98	1.02	1.07	1.43	1.56	1.80	95	2004
Group GL2 (TSH + SD-Sono NAD)	0.74	0.81	0.83	1.14	1.17	1.19	1.76	1.83	1.95	632	2004
L2 Males	0.74	0.83	0.85	1.08	1.12	1.14	1.45	1.49	1.63	332	2004
L2 Females	0.70	0.80	0.83	1.20	1.25	1.30	1.87	1.93	2.28	300	2004
Group GL3 org (NACB crit. for TSH)	0.74	0.82	0.85	1.13	1.15	1.18	1.73	1.79	1.93	448	2004
L3 Males	0.58	0.83	0.85	1.07	1.11	1.14	1.44	1.49	1.73	274	2004
L3 Females	0.69	0.80	0.85	1.20	1.26	1.31	1.87	1.93	2.58	174	2004

(40) T3	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	ng/mL										
Pregnant Women											
1st Trimester	0.99	1.05	1.08	1.42	1.46	1.48	2.14	2.30	2.55	415	2004
2nd Trimester	1.19	1.29	1.40	1.80	1.85	1.89	2.47	2.62	2.86	368	2004
3rd Trimester	1.22	1.35	1.44	1.95	2.02	2.07	2.56	2.62	3.26	169	2004

(41) T3	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	ng/mL										
Group GEL Children, Adolescents											
0 – 6 Days	0.57	0.73	0.79	1.50	1.59	1.79	2.74	3.12	3.25	100	2007
>6 Days ≤3 Months	0.67	0.80	0.85	1.60	1.66	1.78	2.66	2.89	2.98	96	2007
>3 ≤12 Months	0.77	0.86	0.91	1.66	1.69	1.76	2.58	2.72	2.82	101	2007
>1 ≤6 Years	0.86	0.92	0.96	1.67	1.70	1.73	2.40	2.48	2.59	342	2007
>6 ≤11 Years	0.88	0.93	0.97	1.59	1.62	1.65	2.22	2.29	2.41	267	2007
>11 ≤20 Years	0.88	0.91	0.94	1.50	1.53	1.55	2.10	2.16	2.27	473	2007

The data of the different cohorts basically confirm the reference range stated in the package insert. The reference limits are a bit narrower for T3 which reflects local differences and the influence of the inclusion/exclusion criteria for the selection of the cohort.

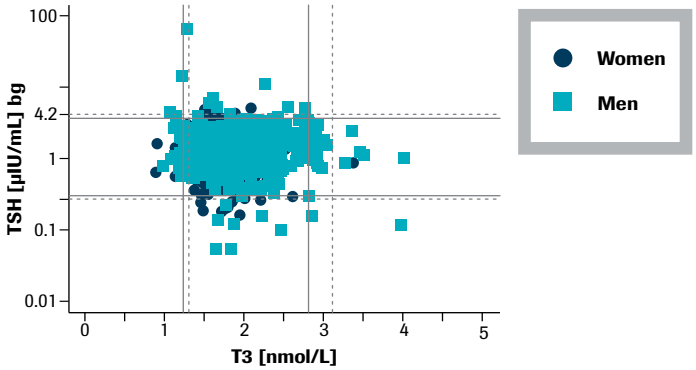
The cohort from Thailand shows a clear decrease of values for the lower reference range (2.5 % quantile) and the median.

A dependency on the increased TBG concentration caused by the intake of contraceptives could be observed. Significantly higher Elecsys® T3 results were seen in the group of women taking contraceptives (GL1 females contraceptives) compared to the group of women not taking contraceptives (GL1 without contraceptives). The results from the group of women not taking contraceptives approximate to those of the group GL1 males. Pregnant women have higher Elecsys® T3 concentrations correlated with the gestational age.

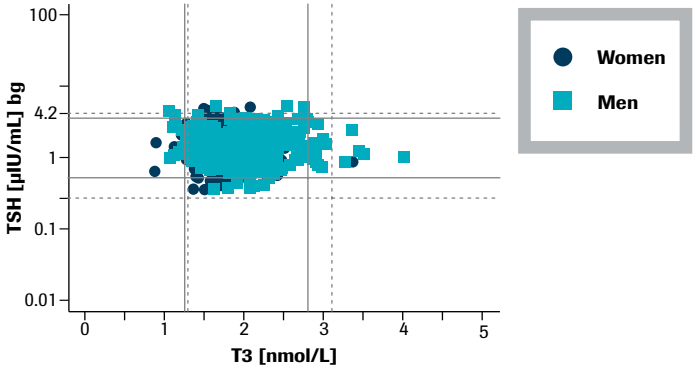
Correlation between Elecsys® TSH and Elecsys® T3 results

In figure 13, each graph shows the Elecsys® T3 results for the subjects of the individual groups GL1, GL2, GL3 and pregnant women, respectively, plotted against the Elecsys® TSH results of the same samples. Samples from male persons are indicated with rectangles and from female persons with circles. The dashed lines correspond to the 2.5 % and 97.5 % quantiles of the valid reference intervals as listed in the package inserts. The continuous lines represent the reference ranges determined in each group which is also stated in the legends. In these graphs, the dependency of the reference values on the applied inclusion/exclusion criteria as well as changes in the values during pregnancy becomes apparent. These results emphasize the importance of applying reference ranges adapted to specific cohorts e.g. trimester specific reference ranges for pregnant women.

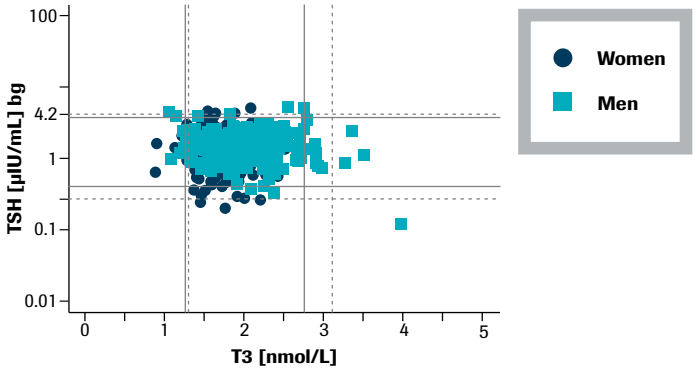
Group GL1 – all blood donners (n = 869 : 445 females, 424 males)



Group GL2 – TSH+Sono NAD (n=643 : 338 females, 305 males)



Group GL3 (TSH NACB) (n=447 : 274 females, 173 males)



Pregnant Women (n=957 : 418 1st trim., 369 2nd trim., 170 3rd trim.)

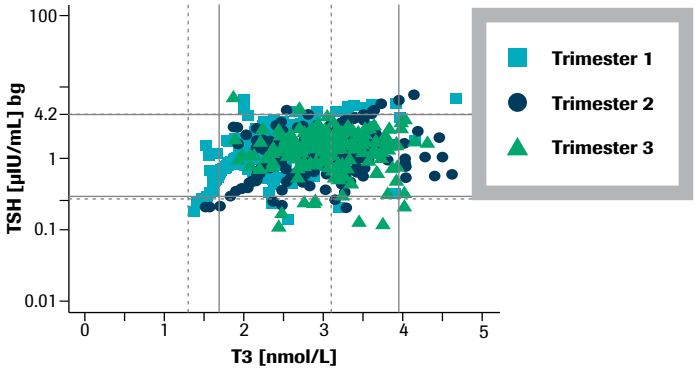


Figure 13: Correlation between Elecsys® T3 and Elecsys® TSH results.

5 Results and Discussion

5.7 Antibodies to TSH receptor – Anti-TSHR

Hyperthyroidism in Graves' disease (autoimmune hyperthyroidism) is typically caused by autoantibodies to the thyroid stimulating hormone receptor (TSHR), and measurement of these TSHR antibodies (TRAb) can be useful in disease diagnosis and management.[3,12,15-17]

TRAb can be classified as stimulating, blocking or neutral depending on their mechanism of action. Despite having actions similar to TSH, TSHR stimulating antibodies are not subject to the negative feedback mechanisms associated with TSH, leading to prolonged activation of the TSHR. This results in the elevated thyroid hormone levels and clinical thyrotoxic state associated with Graves' disease.[18,19]

Indications for TRAb determination include:

- the detection or exclusion of autoimmune hyperthyroidism and its differentiation from disseminated autonomy of the thyroid gland. The presence of TRAb indicates that the patient's thyrotoxicosis is of autoimmune etiology rather than due to toxic nodular goiter.[20,21] Because the aim of treatment for Graves' disease may differ from the treatment of other forms of thyrotoxicosis, an initial TRAb determination is clearly of value.
- monitoring the therapy of Graves' disease patients and prediction of relapse, thereby constituting an important decision-making aid in treatment management. TRAb levels tend to fall during antithyroid drug therapy for Graves' disease. Low levels or the absence of TRAb after a course of drug treatment may indicate disease remission, and therefore the withdrawal of therapy can be considered.[22-24]
- TRAb measurement during the last trimester of pregnancy. Because TRAb are IgG-class antibodies, they cross the placenta and can cause neonatal thyroid disease. The measurement of TRAb during pregnancy in patients with a history of thyroid disease is therefore important in assessing the risk of thyroid disease in the neonate.[25,26]

The tables 42 and 43 below describe the Elecsys® Anti-TSHR results determined for the LIFE adult and child group.

(42) Anti-TSHR	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
				IU/L				
Elecsys® Anti-TSHR package insert					1.22		436	2013
LIFE Adults	<MR	<MR	<MR	1.30	1.36	1.44	4,314	2013
all male patients (sd healthy)	<MR	<MR	<MR	1.33	1.40	1.51	2,572	2013
male <40 years	<MR	<MR	<MR	1.06	1.10	1.83	125	2013
male ≥40 and <70 years	<MR	<MR	<MR	1.33	1.40	1.51	1,889	2013
male ≥70 years	<MR	<MR	<MR	1.27	1.48	1.71	558	2013
all female patients (sd healthy)	<MR	<MR	<MR	1.18	1.26	1.38	1,742	2013
female <40 years	<MR	<MR	<MR	0.95	1.07	1.84	118	2013
female ≥40 and <70 years	<MR	<MR	<MR	1.15	1.21	1.33	1,308	2013
female ≥70 years	<MR	<MR	<MR	1.30	1.51	2.69	316	2013

(43) Anti-TSHR	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
				IU/L				
LIFE Child								
>3 ≤12 Months	<MR	<MR	<MR	<MR	0.94	1.00	19	2016
>1 ≤6 Years	<MR	<MR	<MR	<MR	0.97	1.07	143	2016
>6 ≤11 Years	<MR	<MR	<MR	0.92	1.02	1.14	170	2016
>11 ≤20 Years	<MR	<MR	<MR	0.98	1.05	1.25	145	2016

The upper limits of Elecsys® Anti-TSHR for the cohort of healthy individuals described in the Elecsys® Anti-TSHR package insert is 1.22 IU/L (97.5th percentile). The optimal cutoff of 1.75 IU/L with a sensitivity of 96 % and a specificity of 99 % has been determined in the study described in the package insert by calculating the receiver operating characteristic (ROC) curve. The results generated in both studies are comparable and did not reveal impact of age and gender.

5 Results and Discussion

5.8 Antibodies to thyroid peroxidase – Anti-TPO

Elevated serum titers of antibodies to TPO are found in several forms of thyroiditis caused by autoimmunity.[31,32]

High Anti-TPO titers are found in up to 90 % of patients with chronic Hashimoto's thyroiditis. In Graves' disease, 70 % of the patients have an elevated titer.[32-34] Although the sensitivity of the diagnostic procedure can be increased by simultaneously determining other thyroid antibodies (Elecsys® Anti-Tg, Elecsys® Anti-TSHR), a negative finding does not rule out the possibility of an autoimmune disease. The magnitude of the antibody titer does not correlate with the clinical activity of the disease.[33-35] Initially elevated titers can become negative after lengthy periods of illness or during remission. If antibodies reappear following remission, then a relapse is probable.[54]

The determination of Elecsys® Anti-TPO together with Elecsys® TSH is recommended:

- for the detection of suspected dysfunction, especially regarding the long-term risk of the development of a thyroid disease
- during and after pregnancy
- to clarify subclinical hypothyroidism

The tables 44 to 47 below describe the Elecsys® Anti-TPO reference ranges determined in different studies for: 1. adults, separated into male and female and age, 2. pregnant women, separated into the 3 trimesters and 3. children, separated by age. All confidence intervals were computed with 95 % confidence level.

(44) Anti-TPO	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (95 % quant.)	95 % quant.	95p CI high (95 % quant.)	N	Year of publ.
				IU/mL				
Elecsys® Anti-TPO package insert					34.00		208	2000
Group GL1 (all blood donors)	13.7	14.1	14.7	92.5	186	287	870	2004
L1 Males all	13.0	13.5	14.1	32.9	37.1	63.4	445	2004
L1 Females all	14.3	14.9	15.7	227	312	422	425	2004
Group GL2 (TSH + SD-Sono NAD)	13.3	14.0	14.6	62.3	167	297	632	2004
L2 Males	12.8	13.4	14.1	31.7	37.0	63.4	332	2004
L2 Females	13.7	14.7	15.4	194	308	426	300	2004
Group GL5 (NACB crit. for a-TPO) 90 % cent.	11.5	12.6	14.5	23.8	28.2	32.7	80	2004
LIFE Adults	10.3	10.5	10.7	76.0	85.8	97.7	6,080	2013
all male patients (sd healthy)	10.0	10.2	10.5	42.2	48.1	62.6	3,418	2013
male <40 years	8.60	9.45	10.2	29.7	93.1	388	168	2013
male ≥40 and <70 years	10.30	10.50	10.7	43.9	52.4	68.9	2,515	2013
male ≥70 years	9.00	9.50	9.98	30.3	38.4	46.9	735	2013
all female patients (sd healthy)	10.6	10.9	11.2	111	137	173	2,662	2013
female <40 years	9.30	10.4	11.6	89.4	304	384	173	2013
female ≥40 and <70 years	10.9	11.2	11.6	115	146	187	2,044	2013
female ≥70 years	9.10	9.60	10.10	54.9	78.7	125	445	2013

(45) Anti-TPO	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (95 % quant.)	95 % quant.	95p CI high (95 % quant.)	N	Year of publ.
	IU/mL							
Pregnant Women								
1st Trimester	10.8	11.3	11.8	47.0	64.4	119	410	2004
2nd Trimester	9.98	10.5	11.4	30.1	50.8	101	362	2004
3rd Trimester	12.4	13.3	14.6	40.0	123	171	165	2004

(46) Anti-TPO	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (95 % quant.)	95 % quant.	95p CI high (95 % quant.)	N	Year of publ.
	IU/mL							
Group GEL Children, Adolescents								
0 – 6 Days	5.67	6.23	10.5	34.7	66.0	87.1	91	2007
>6 Days ≤3 Months	5.97	6.47	8.96	28.7	38.5	43.8	107	2007
>3 ≤12 Months	6.21	6.69	8.19	25.5	27.5	29.4	123	2007
>1 ≤6 Years	6.82	7.26	7.87	20.0	21.4	25.4	344	2007
>6 ≤11 Years	7.71	8.15	8.58	22.1	24.3	31.2	249	2007
>11 ≤20 Years	8.66	9.12	9.54	27.2	31.9	42.1	462	2007

(47) Anti-TPO	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (95 % quant.)	95 % quant.	95p CI high (95 % quant.)	N	Year of publ.
	IU/mL							
LIFE Child								
>3 ≤12 Months	5.69	6.05	6.87	11.7				
>1 ≤6 Years	8.05	8.79	9.60	16.3	12.4	14.5	83	2016
>6 ≤11 Years	8.01	8.65	9.20	17.3	18.1	20.9	190	2016
>11 ≤20 Years	8.22	8.72	9.70	16.7	19.8	24.3	257	2016

The selection of inclusion and exclusion criteria clearly influences the cutoff levels. The non-selected group of blood donors (GL1), group GL2 and LIFE adult show significant higher values. These results are caused by the group of women, who showed essentially higher values.

The group GL5 selected according to the NACB criteria for the reference interval determination of Elecsys® Anti-TPO and Elecsys® Anti-Tg (e.g. only males <30 years) show with 28.1 IU/mL a lower cutoff as compared to that of Elecsys® Anti-TPO. The cutoff of 34 IU/mL currently stated in the package insert (mat. No.: 06368590 190 V4.0 and 07026935 190 v1.0 status June 2018) is close to the upper limit of the 95 % confidence interval of 32.68 IU/mL in the GL5 cohort.

The cutoff published by EQALIS is the lowest with a 95 % quantile limit of 19 IU/mL.

In pregnant women the Elecsys® Anti-TPO concentrations are slightly higher in the 3rd trimester. There is no significant difference between the three trimesters.

5 Results and Discussion

5.9 Antibodies to thyroglobulin – Anti-Tg

The Elecsys® Anti-Tg assay is used in monitoring the course of Hashimoto's thyroiditis and for the differential diagnosis:

- cases of suspected autoimmune thyroiditis of unknown origin with negative Anti-TPO test results
- Graves' disease without lymphocytic infiltration
- to rule out interference by Tg-autoantibodies in the Tg test

Elevated serum concentrations of antibodies against Tg are found in subjects with autoimmunity-based thyroiditis.[37,38] High concentrations of Anti-Tg together with Anti-TPO are present in most patients with chronic lymphocytic-infiltrative thyroiditis (Hashimoto's disease).[38] The frequency of thyroglobulin antibodies is approximately 50 – 80 % in subjects with autoimmune-thyroiditis, including Hashimoto's disease, and approximately 30 – 50 % in individuals with Graves' disease.[38-41] The Anti-Tg assay can also provide useful information for:

- monitoring the course of Hashimoto's thyroiditis
- differential diagnosis

This includes cases of suspected autoimmune thyroiditis of unknown origin with negative Anti-TPO test results[34,42] and to distinguish Hashimoto's thyroiditis from nontoxic nodular goiter or from other forms of thyroiditis.[39]

Anti-Tg has also been reported as a useful surrogate diagnostic marker for differentiated thyroid cancer when serum Tg is negative[43] and for ruling out interference by Tg autoantibodies when measuring serum Tg using a Tg test.[45,56]

The tables 48 to 51 below describe the Elecsys® Anti-Tg reference ranges determined in different studies for: 1. adults, separated into male and female and age, 2. pregnant women, separated into the 3 trimesters and 3. children, separated by age. All confidence intervals were computed with 95 % confidence level.

(48) Anti-Tg	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (94 % quant.)	94 % quant.	95p CI high (94 % quant.)	N	Year of publ.
	IU/mL							
Elecsys® Anti-Tg package insert					115		392	2001
Group GL1 (all blood donors)	17.2	17.6	18.2	313	400	455	870	2004
L1 Males all	16.2	16.7	17.3	36.6	67.3	188	445	2004
L1 Females all	18.1	18.9	20.3	443	492	623	425	2004
Group GL2 (TSH + SD-Sono NAD)	16.4	16.9	17.6	218	350	415	632	2004
L2 Males	15.7	16.3	16.9	33.7	72.0	248	332	2004
L2 Females	17.2	18.2	19.2	402	436	531	300	2004
Group GL5 (NACB crit. for a-TPO) 90 % cent.	18.6	23.7	25.0	25.0	33.5	98.1	80	2004
LIFE Adults	17.6	17.8	18.0	172	200	245	5,047	2013
all male patients (sd healthy)	16.7	17.0	17.3	57.5	66.7	87.7	2,863	2013
male <40 years	17.8	19.6	21.4	76.8	175	388	145	2013
male ≥40 and <70 years	16.9	17.2	17.6	57.7	70.1	106	2,110	2013
male ≥70 years	15.3	15.9	16.3	29.4	38.0	63.7	608	2013
all female patients (sd healthy)	18.7	19.1	19.5	331	385	412	2,184	2013
female <40 years	18.0	19.8	21.4	221	369	555	139	2013
female ≥40 and <70 years	19.0	19.5	20.0	340	397	422	1,667	2013
female ≥70 years	16.6	17.5	18.0	141	273	415	378	2013

(49) Anti-Tg	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (94 % quant.)	94 % quant.	95p CI high (94 % quant.)	N	Year of publ.
	IU/mL							
Pregnant Women								
1st Trimester	18.1	18.7	19.8	333	398	511	418	2004
2nd Trimester	16.0	16.5	17.4	85.2	139	195	369	2004
3rd Trimester	17.0	17.4	17.9	37.2	62.1	370	170	2004

(50) Anti-Tg	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (94 % quant.)	94 % quant.	95p CI high (94 % quant.)	N	Year of publ.
	IU/mL							
Group GEL Children, Adolescents								
0 – 6 Days	19.5	25.9	31.0	107	170	255	93	2007
>6 Days ≤3 Months	16.7	20.1	22.7	82.3	100	123	110	2007
>3 ≤12 Months	15.1	17.1	18.5	63.4	72.2	85.8	120	2007
>1 ≤6 Years	13.7	14.4	14.9	45.8	55.1	73.0	346	2007
>6 ≤11 Years	13.4	13.8	14.2	47.6	60.1	83.8	254	2007
>11 ≤20 Years	13.7	14.1	14.4	60.6	75.8	107	465	2007

(51) Anti-Tg	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (94 % quant.)	94 % quant.	95p CI high (94 % quant.)	N	Year of publ.
	IU/mL							
LIFE Child								
>3 ≤12 Months	12.8	14.1	15.7	19.4	21.9	26.2	22.0	2016
>1 ≤6 Years	15.3	16.1	17.7	24.4	27.3	30.0	160	2016
>6 ≤11 Years	15.6	16.3	17.3	25.2	29.7	45.9	197	2016
>11 ≤20 Years	15.2	16.5	17.6	33.3	71.7	104	170	2016

The non-selected group of blood donors, GL1, the group GL2 and the group LIFE adult show significant higher values compared to GL5. These results are caused by the group of women, which showed essentially higher values compared to the group of males.

Group GL5 was selected according to the NACB criteria for the reference interval determination of Elecsys® Anti-TPO and Elecsys® Anti-Tg (e.g. only males <30 years). GL5 shows for Elecsys® Anti-Tg with 33 IU/mL clearly a lower cutoff as compared to that of Elecsys® Anti-Tg package insert (115 IU/mL). This cutoff is still not covered by the upper limit of the 95 % quantile in the group GL5 (98 IU/mL). This finding reflects the influence of cohort selection.

In pregnant women the Elecsys® Anti-Tg concentrations are significantly higher in the 1st trimester as compared to the 2nd and 3rd trimester. The results for the 2nd and the 3rd trimester are nearly identical.

5 Results and Discussion

5.10 Thyroglobulin – Tg

Synthesis of T3 and T4 from Thyroglobulin (Tg) is regulated by TSH, intrathyroidal iodine levels and the presence of thyroid-stimulating immunoglobulins. During synthesis of Tg by the thyrocytes and the transport of Tg to the follicles, small quantities of the protein can pass into the bloodstream. Accordingly, low concentrations of Tg can be found in the blood of healthy individuals not suffering from thyroid diseases.[46]

Elevated Tg concentrations have been reported in many different thyroid conditions such as Hashimoto's disease, Graves' disease, thyroid adenoma, and thyroid carcinoma. The determination of Tg can also be helpful to distinguish between subacute thyroiditis and factitious thyrotoxicosis. In cases of congenital hypothyroidism the determination of Tg can be used to differentiate between the complete absence of the thyroid gland and thyroid hypoplasia or other pathological conditions.[13,47,48]

The main application of Tg testing is the post-operative follow-up of patients with differentiated thyroid carcinoma (DTC). A global rise in the prevalence of DTC has resulted in higher numbers of thyroidectomized patients who require lifelong monitoring for persistent or recurrent disease.[48,49] As the thyroid gland is the only known source of Tg, the serum Tg level will drop to a very low or undetectable concentration after total or near-total thyroidectomy and successful radioiodine ablation of the residual thyroid tissue. Detectable levels of serum Tg after total thyroidectomy are indicative of persistent or recurrent DTC. As a consequence significantly increasing Tg levels are interpreted as a sign of recurrence of the disease.[50-56] In patients who have undergone a partial thyroidectomy Tg levels will still be measurable depending on how much tissue is remaining after surgery.

The table 52 below describes the Elecsys® Tg results determined in the MCE study.

(52) Tg	95p CI low (2.5 % quant.)	2.5 % quant.	95p CI high (2.5 % quant.)	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
						ng/mL					
Elecsys® Tg package insert		3.5						77		478	2012

5 Results and Discussion

5.11 Calcitonin – hCT

Calcitonin is metabolized in the liver and kidney and regulated by serum calcium levels. Physiologically hCT has effects on calcium and phosphorus metabolism. It is an inhibitor of bone resorption to prevent bone loss at times of calcium stress (e.g. pregnancy, lactation and growth).[57,58]

The most prominent clinical syndrome associated with a disordered hypersecretion of hCT is the medullary thyroid carcinoma (MTC), a tumor of the calcitonin secreting cells of the thyroid, which comprises 5 – 10 % of all thyroid cancers. 75 – 80 % of cases occur sporadically and the remainder as an autosomal dominant trait. MTC Management Guidelines were developed by the American Thyroid Association and recommend calcitonin measurements in the risk stratification / selection of treatment in inherited MTC and in the evaluation and treatment post thyroidectomy.[59,60] These recommendations were endorsed by the European Thyroid Association and extended by an European Panel of Experts to routine measurement of serum calcitonin in patients with thyroid nodules.[61] Moderately elevated calcitonin levels can be falsely positive for either technical reasons or the presence of other rare pathological conditions (i.e. other neuroendocrine tumors, hyperparathyroidism, renal failure etc.). Therefore, the European Panel of Experts recommends that subjects with elevated basal calcitonin undergo a stimulation test, either by injection of pentagastrin or a rapid infusion of calcium. Most MTCs respond with a significant increase of hCT levels upon stimulation.[62,63]

The table 53 below describes the hCT results determined in the MCE study for Calcitonin.

The serum Elecsys® hCT levels for adult men are significantly higher compared to adult women whereas smoking may lead to an additional increase in serum calcitonin levels. These finding correlate with the literature.[64-66]

(53) hCT	95p CI low (95 % quant.)	95 % quant.	95p CI high (95 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
	pg/mL							
Elecsys® hCT package insert female				5.17	6.40	9.82	193	2013
female smoker	5.91	6.81	7.49	5.91	7.49	7.49	27	2013
female non-smoker	3.16	3.84	6.37	3.58	4.30	6.75	142	2013
Elecsys® hCT package insert male				8.31	9.52	14.3	162	2013
male smoker	8.31	8.47	10.9	8.31	10.9	10.9	29	2013
male non-smoker	6.73	7.72	14.3	7.22	9.52	14.32	102	2013

The table 54 below describes the hCT results determined for children in the LIFE child study.

(54) hCT	95p CI low (50 % quant.)	50 % quant.	95p CI high (50 % quant.)	95p CI low (97.5 % quant.)	97.5 % quant.	95p CI high (97.5 % quant.)	N	Year of publ.
				pg/mL				
LIFE Child female								
>3 ≤12 Months	7.07	10.7	13.9	20.6	26.3	27.8	23	2016
>1 ≤6 Years	2.55	3.07	3.98	7.08	8.68	11.0	70	2016
>6 ≤11 Years	1.97	2.29	2.66	5.84	7.42	8.12	97	2016
>11 ≤20 Years	<MR	<MR	0.99	2.01	3.96	6.53	70	2016
LIFE Child male								
>3 ≤12 Months	8.65	10.6	14.0	21.5	26.6	28.4	37	2016
>1 ≤6 Years	3.33	4.12	5.54	8.59	13.0	22.7	76	2016
>6 ≤11 Years	3.31	4.01	4.71	7.38	9.81	12.1	101	2016
>11 ≤20 Years	0.67	1.12	2.06	2.14	4.11	5.45	76	2016

Elecsys® hCT results for LIFE child female and male do not show significant differences from each other. In general, the Elecsys® hCT levels are relatively high in infants, decline rapidly and are relatively stable from childhood (about the age of 11) through adult life.

6 Concentrations of Elecsys® TSH, Elecsys® FT4 and Elecsys® FT3 in the serum of euthyroid inpatients and outpatients

A key issue for clinicians is the extent to which they can reliably apply the algorithms of thyroid laboratory diagnostics to inpatients and outpatients with a broad spectrum of nonendocrine disease. The aim of this article is to identify the laboratory diagnostic characteristics that need to be taken into account when excluding thyroid dysfunction in intensive care unit (ICU) patients and in patients with heart disease (HD), rheumatic disease (RD), type 2 diabetes mellitus (DM), and renal failure (RF). We present and compare the general and group-specific determinants of serum thyroid-stimulating hormone (TSH), free thyroxine (FT4) and free triiodiothyronine (FT3) concentrations observed in a Leipzig University Hospital study in 120 patients selected per specified disease and also as recorded in the literature.

1. Serum Elecsys® TSH determinants

The group distribution of TSH concentrations (Figure 14) shows clearly lower values in ICU and RD patients than in the other groups.

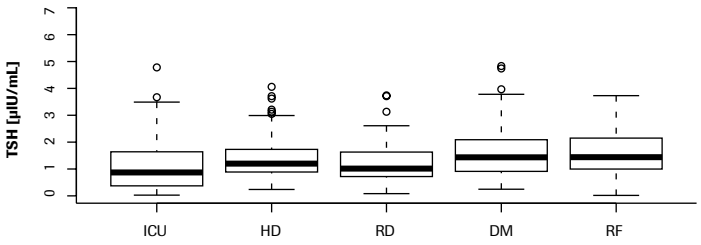


Figure 14: Boxplots showing Elecsys® TSH results in different patient groups.

Median concentrations differed only slightly in the HD, DM, and RF groups. Possible determinants of Elecsys® TSH concentrations in the individual groups include:

General determinants

Factors that lower TSH levels comprise acute illness (infection etc), severe (preterminal) disease progression (like sepsis), surgery, fasting, and smoking. Other factors are mainly pharmacologic: dopamine, heparin, calcium channel blockers, and nonsteroidal antiinflammatory drugs (NSAIDs).

Cirrhosis of the liver and hypercholesterolemia can increase TSH values, as can stress and the menopause. Drugs with a similar effect include lithium, beta-blockers, and opioids.

Group-specific determinants

- **ICU**
  Euthyroid sick syndrome (abnormal thyroid function results in a nonthyroid illness setting) is common in ICU patients. Lifethreatening illness is associated with central down-regulation of TSH with a simultaneous decrease in FT3 and, above all in the final stages, in FT4.

- **RD**
  Glucocorticoids and NSAIDs lower basal TSH levels, no doubt accounting for the tendency towards lower values in this group.

- **DM**
  TSH levels were little changed. Relatively high levels, approaching 5 mU/L, can be found in individual patients, possibly associated with hypercholesterolemia.

- **RF**
  The effect of dialysis on TSH is disputed, but levels may increase in some cases.

2. Serum Elecsys® FT4 determinants

The group distribution of Elecsys® FT4 concentrations (Figure 15) shows a clear decrease in ICU patients, but also a trend towards lower values in RF patients compared to the HD, DM, and RD groups.

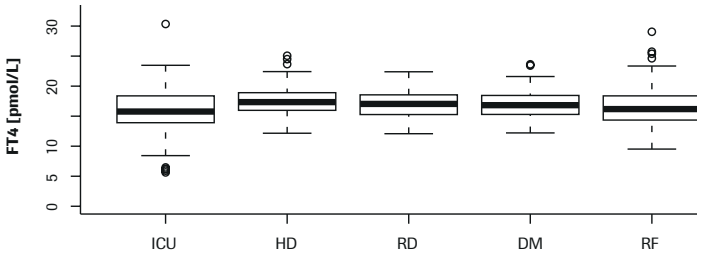


Figure 15: Boxplots showing Elecsys® FT4 results in different patient groups.

In all groups, the expected number of data points for a more or less relevant number of Elecsys® FT4 results exceeded the 97.5 % quantile in blood donors, namely 22 pmol/L. The increase was seen mainly in the ICU, HD, and KI groups. In addition, values varied widely in ICU and RF patients. Possible determinants of FT4 values in the individual groups include:

General determinants

Diseases associated with increased FT4 levels comprise anorexia nervosa, decompensated cirrhosis of the liver, and acute psychiatric illness. Drugs with a similar effect include beta-blockers, heparin, aspirin/NSAIDs, and also furosemide, which blocks the binding of T4 to plasma proteins.

Decreased serum FT4 values have been observed in response to drugs such as fenclofenac, furosemide, lithium, propranolol, phenobarbital, phenylbutazone, and phenytoin.

Group-specific determinants

- **ICU**
  Euthyroid sick syndrome is a common cause of low FT4 levels in patients with life-threatening or critical illness. Central downregulation of TSH leads to a decrease in FT3 and, above all in the final stages, in FT4.
- **HD**
  Individual drugs can affect thyroid hormone concentrations in these polymedicated patients. Thus beta-blockers such as propranolol, that decrease the peripheral conversion of T4 to T3, may account for the higher FT4 levels in some patients in this group.
- **RF**
  Chronic RF decreases FT3 and FT4 concentrations, accounting for the trend towards lower levels in this group. An increase in FT4 is observed after hemodialysis, which may be associated with heparin administration.

3. Serum Elecsys® FT3 determinants

The group distribution of FT3 concentrations (Figure 16) shows a clear decrease in ICU patients.

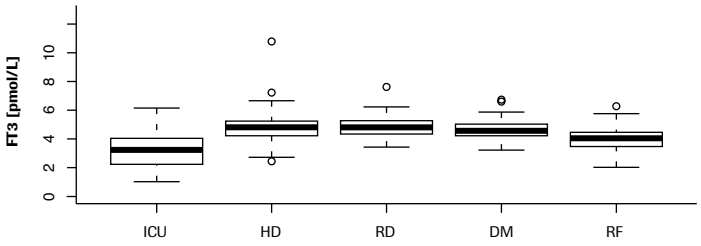


Figure 16: Boxplots showing Elecsys® FT3 results in different patient groups.

Values also tended to be lower in the RF group than in patients with HD, RD, or DM. Individual HD sera showed a tendency towards decreased concentrations. Possible determinants of FT3 values in the individual groups include:

General determinants

Factors lowering FT3 concentrations comprise acute illness (like infection), severe (preterminal) disease progression (like sepsis), advancing age, chronic RF, and surgery. Drugs with a similar effect include glucocorticoids and beta-blockers.

Aspirin/NSAIDs increase FT3 by displacing the hormone from its binding to plasma proteins. Posttraumatic stress may also increase FT3 levels.

Group-specific determinants

- **ICU**
  Euthyroid sick syndrome is a common cause of low FT3 levels in patients with life-threatening or critical illness. Central downregulation of TSH leads to a decrease in FT3 and, above all in the final stages, in FT4.

- **HD**
  Individual drugs can affect thyroid hormone concentrations in these polymedicated patients. Thus beta-blockers such as propranolol, that decrease the peripheral conversion of T4 to T3, may account for the lower FT3 levels in some patients in this group.

- **RF**
  Chronic RF lowers free hormone levels.

4. Summary

Pathological concentrations of thyroid parameters TSH, FT4 and FT3 in the serum of nonthyroid patients generally indicate overt or subclinical thyroid dysfunction. However, patients' underlying disease and/or drug treatments, as well as other factors both preanalytical and analytical, can also produce pathological TSH, FT4 and FT3 concentrations without signifying thyroid dysfunction. In addition, it should be borne in mind that reference ranges are determined using the 2.5 % and 97.5 % quantile of the hormone concentrations in euthyroid subjects. Given this statistical background, tests on the sera of 100 euthyroid individuals will yield 2 – 3 samples with levels above or below the reference range. We studied the distribution of thyroid hormone concentrations in severely ill but clinically euthyroid patients in an ICU, and in patients with HD, RD, DM, and RF. The results can be summarized as follows:

1 In most critically ill patients, e.g. after major surgery or with severe infection, TSH and FT3 levels were decreased, as also were FT4 levels in the more serious cases (euthyroid sick syndrome).
2 In HD patients TSH and FT4 concentrations were normal, with FT3 tending to be lower in individual cases, presumably because drug-induced (beta-blockers).
3 In RD patients, standard synthetic glucocorticoid and NSAID therapy no doubt accounted for the decreased serum TSH concentrations in some cases.
4 In DM patients, almost all thyroid hormone parameters were in the reference range. Hypercholesterolemia may explain the slight increase in TSH values in rare cases.
5 Some RF patients exhibited decreased FT3 or FT4 values with normal TSH concentrations.
6 A clear number of patients from all groups exhibited FT4 concentrations exceeding the upper reference range (97.5 % quantile).

In most cases, diagnostic algorithms based on serum TSH, FT3 and FT4 determination can be relied on to exclude thyroid disease in inpatients and outpatients with a broad spectrum of nonendocrine disease. However, specific illness-related determinants of the individual hormone concentrations must be taken into consideration when interpreting laboratory values.

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Patient group	TSH (µIU/mL)		FT4 (pmol/L)		FT3 (pmol/L)	
	Median	Quantile	Median	Quantile	Median	Quantile
		2.5 – 97.5		2.5 – 97.5		2.5 – 97.5
ICU (n=111)	0.80	0.05 – 3.33	15.8	6.19 – 22.75	3.13	1.34 – 5.56
HD (n=100)	1.20	0.41 – 3.21	17.3	13.91 – 22.42	4.80	2.73 – 6.48
RD (n=99)	1.01	0.20 – 3.13	17.1	13.12 – 21.63	4.81	3.46 – 6.23
DM (n=109)	1.41	0.30 – 3.97	16.9	12.91 – 23.42	4.57	3.73 – 5.88
RF (n=112)	1.40	0.20 – 3.35	16.1	11.30 – 22.80	4.02	2.62 – 5.32

7 Conclusion

This brochure provides a rather complete survey of results on the reference intervals of the Elecsys® thyroid parameters. The data evaluated embrace the time-span from launch of the Elecsys® systems in 1996 until 2016.

This is an overview on the performed studies:

- 1996: Elecsys® TSH, Elecsys® T3
- 1998: Elecsys® FT4, Elecsys® T4, Elecsys® Tuptake
- 2000/2001: Elecsys® Anti-Tg, Elecsys® Anti-TPO
- 2003: Elecsys® FT3
- 2003: additional reference ranges for adults, children and pregnant women for Elecsys® TSH, Elecsys® FT4, Elecsys® FT3, Elecsys® T4, Elecsys® T3, Elecsys® Anti-TPO, Elecsys® Anti-Tg, Elecsys® Tuptake
- 2007: extension of data for children according to the in 2003 investigated thyroid parameters
- 2012: Elecsys® Tg
- 2013: Elecsys® hCT
- 2016: additional reference ranges for adults and children for Elecsys® TSH, Elecsys® FT4, Elecsys® FT3, Elecsys® Anti-TSHR, Elecsys® Anti-TPO, Elecsys® Anti-Tg, Elecsys® hCT

In 2004, with the launch of the Elecsys FT3® II, the first reference interval brochure has been published. In addition, a new reference range study was initiated for all Elecsys® thyroid parameters to investigate whether there might have been any significant shifts in the ranges obtained earlier. A further goal was to characterize in great detail the most important influencing factors on thyroid parameters. Allowing for minor changes in the estimated reference values due to the particular compositions of the respective reference groups, we observed a remarkable stability of the reference intervals of the Elecsys® thyroid parameters during the course of time. In fact, there are only very few cases where the established reference value of the package insert was not covered by the confidence interval of the newly calculated quantile.

With a cohort of pregnant women additional statements could be made on the ranges for all thyroid parameters according to trimesters. The ranges, especially those for thyroid autoantibodies, must be assessed under consideration of the information available on the pregnant women.

In the very well characterized group of blood donors from Leipzig, Germany, age and sex of the subjects have been identified as the most important influencing factors. Within the females, the use of oral contraceptives was another decisive influencing factor of the values.

In 2007, a new study in Leipzig, Germany, was performed to update and complete the children reference range values. The brochure has been updated accordingly.

In the group of in- and outpatients described in chapter 6, the influence of non-thyroidal disease and drugs has been investigated also reflecting that certain conditions may have an impact on the TSH, FT4 and FT3 results.

In 2016, new reference range studies for most of the Elecsys® thyroid parameters have been initiated in the context of the LIFE study (http://life.uni-leipzig.de) in Leipzig, Germany. The studies supported another investigation whether there might have been any significant shifts in the ranges obtained earlier. The results confirmed – as already in the studies performed in 2003 and 2007 – a remarkable stability of the reference intervals for the Elecsys® thyroid parameters during the course of time, also ruling out reference shifts due to generation changes for Elecsys® FT3 and Elecsys® FT4 assays.

In the LIFE child cohort, reference ranges for children show quite dramatic changes in the levels of the most thyroid parameters during early childhood. This has been already shown and reported in the previous studies for children in Erlangen and Leipzig, Germany. Therefore, reporting of age related reference ranges for at least TSH, FT3, T3 and hCT are highly recommended.

With the data generated in the LIFE adult cohort, reference ranges for a broader age range up to 80 years is available, now. Age dependency could be observed especially for TSH and FT3 which leads to the recommendation of applying age specific reference ranges to patients.

The LIFE adult cohort allowed the computation of reference ranges for the common group of patients under L-thyroxine supplementation pointing out the effect of this medication on reference ranges.

In general, laboratories should check the reference ranges for transferability. This reference interval brochure for adults, children and pregnant women shall support a more precise and accurate diagnosis of thyroid conditions, enabling laboratories to apply group specific reference intervals to offer high diagnostic value to their customers and patients.

Short description

Detailed description can be found in chapter.

Group GEL
Newborns, infants, children and adolescents in age 0 – 20 years, Erlangen and Leipzig, Germany 2003, 2004, 2007.

Group GL1
Blood donors, men and women, no exclusion criteria. Adults Leipzig, Germany 2003, 2004.

Group GL2
Normal thyroid ultrasound (volume and structure), ADVIA Centaur TSH within reference range. Adults Leipzig, Germany 2003, 2004.

Group GL3
Inclusion and exclusion criteria according to Guideline 22 of the National Academy of Clinical Biochemistry (NACB), USA 2002.[3] Adults Leipzig, Germany 2003, 2004.

Group GL5
Inclusion and exclusion criteria according to Guideline 33 of the NACB, USA 2002[3]. Adults Leipzig, Germany 2003, 2004.

Group P
Apparently healthy women in the 1st, 2nd and 3rd trimester of pregnancy without complication. Essen and Hamburg, Germany.

LIFE Adult
apparently healthy adults, Leipzig, Germany 2013.

LIFE Child
apparently healthy children, Leipzig, Germany 2016.

Group GHH
apparently healthy adults, Hamburg, Germany 2003.

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