AIA-360 Assay Specifications
ST FT4 Test Code 075

No.	Item	Data
1	Calib. Req	Show
2	Cal 1	0 ng/dL
3	Cal 2	0.4 ng/dL (example)
4	Cal 3	1.0 ng/dL (example)
5	Cal 4	2.5 ng/dL (example)
6	Cal 5	4.0 ng/dL (example)
7	Cal 6	9.0 ng/dL (example)
8	Cal Lot L	
9	Cal Lot R	
10	Unit	ng/dL
11	Smpl. Vol	10
12	Dil. Vol	140
13	Assay L	0.10
14	Assay H	8.0
15	Ref. L	
16	Ref. H	
17	Decimal	2
	Visible only in Test Mode	
18	Test Name	#FT4
19	Calib. No	6
20	Calib. Mul	3
21	Calib. Equ	5
22	Calib. CV	90
23	Assay Prtl	1
24	Factor1 A	1.000000
25	Factor1 B	0.000000
26	Factor2 A	1.000000
27	Factor2 B	0.000000
28	V. Conc	0.000000
29	G Origin	0.000000

AIA-600II Assay Specifications
ST FT4 Test Code 075

Screen	Item	Data
Screen 1		
	Lot	***Enter current cal. lot no.
	Cal 1	0 ng/dL
	Cal 2	0.4 ng/dL (example)
	Cal 3	1.0 ng/dL (example)
	Cal 4	2.5 ng/dL (example)
	Cal 5	4.0 ng/dL (example)
	Cal 6	9.0 ng/dL (example)
Screen 2		
	Name	#FT4
	Unit	ng/dL
	Smpl	10
	Dil	140
	2Reag	0
	Code	0
	Assay Range Low	0.1
	Assay Range High	8.0
	Reference Range Low	
	Reference Range High	
	DP (No. of decimal points)	2
Screen 3		
	Code	46
	No. (Calibrators)	6
	Mul. (Replicates)	3
	Equ	5
	CV (Calibration curve stability)	90
	STAT (Analyte status)	0
	PRCL (Assay Protocol)	1
Screen 4		
	Dilution Factors:	
	SP1 (Specimen 1)	1
	SP2 (Specimen 2)	1
	CAL	1
	CTRL	1
	CODE (SDS code)	0
	PR (Dilution mode)	3
	Pretreatment:	
	SMPL (Pretreatment sample volume)	0
	VOL1 (Pretreatment 1 volume)	0
	VOL2 (Pretreatment 2 volume)	0
	CODE (Pretreatment code)	0

AIA-900 Assay Specifications
ST FT4 Test Code 075

No.	Item	Data
1	Code	75
2	ACT	0
3	Analyte	#FT4
4	Lot	***Enter current cal lot no.
5	CAL 1	0 ng/dL
6	CAL 2	0.4 ng/dL (example)
7	CAL 3	1.0 ng/dL (example)
8	CAL 4	2.5 ng/dL (example)
9	CAL 5	4.0 ng/dL (example)
10	CAL 6	9.0 ng/dL (example)
11	Cal lot L	
12	Cal lot R	
13	Unit	ng/dL
14	Decimal	2
15	Assay Low	0.1
16	Assay High	8
17	Reference Low	
18	Reference High	
19	Reschedule Low	0.1
20	Reschedule High	8
21	Factor A	1
22	Factor B	0
23	Sample Volume	10
24	Diluent Volume	140
25	2 Step reagent dispensing volume	0
26	Calibration code	46
27	CAL. No.	6
28	CAL. MUL.	3
29	CAL. EQU.	5
30	CAL. CV	90
31	DIL. SP1	1
32	DIL. SP2	1
33	DIL. CAL. (Calculation of dil ratio of conc.)	1
34	DIL. CNTL.	1
35	DIL. DO	1
36	DIL. AH.	1
37	DIL. CALC.	1
38	DIL. CODE	0
39	DIL. NAME	
40	DIL. PRTY	3
41	PRE. SPVOL (Vol. of pretreated sample)	0
42	PRE. 1VOL (Vol. of pretreatment sol-1)	0
43	PRE. 2 VOL (Vol. of pretreatment sol-2)	0
44	PRE. CODE (pretreated sol. code)	0
45	PRE. NAME (pretreated sol. name)	0
46	Protocol	1
47	SYS. F_A	1
48	SYS. F_B	0
49	V. CONC.	0
50	G. ORIGIN	0

AIA-1800 Assay Specifications
ST FT4 Test Code 075

No.	Item	Data
1	Unit	ng/dL
2	Decimal places	2
3	Reference low	
4	Reference high	
5	Reschedule low	0.10
6	Reschedule high	8.0
7	Assay range low	0.10
8	Assay range high	8.0
9	Specimen diluent code	
10	Specimen diluent name	
11	Dilution factor for Sp. 1	1
12	Dilution factor for Sp. 2	1
13	Dilution factor for Control	1
14	Default multiplier for DO	1
15	Default multiplier for >H	1
16	Factor A	1.00000e+000
17	Factor B	0.00000e+000
18	Calibration code	5
19	Calibrator replicates	3
20	Calibrator lot	***Enter current cal. lot no.
21	Calibrator Concentration	
22	Cal - 01	0 ng/dL
23	Cal - 02	0.4 ng/dL (example)
24	Cal - 03	1.0 ng/dL (example)
25	Cal - 04	2.5 ng/dL (example)
26	Cal - 05	4.0 ng/dL (example)
27	Cal - 06	9.0 ng/dL (example)
28		
29		
30		
31		
32		
33		
34	Dilution factor for calibrator	1
35	Calibration period	90
36	Incubation time (10/40)	10
37	Assay protocol	1
38	Specimen volume	10
39	Diluent volume	140
40	Conjugate volume	0
41	Diluted conjugate volume	0
42	Pretreatment	0
43	Pretreatment specimen volume	0
44	Pretreatment Reagent code	0
45	Pretreatment Reagent name	0
46	Pretreatment Reagent 1 volume	0
47	Pretreatment Reagent 2 volume	0
48	System Factor	1
49	Calibration Code Check	046
50	Virtual Concentration	0.0
51	Graph Origin	0.0
52	Calculation with Dilution Factor	Yes

AIA-2000 Assay Specifications
ST FT4 Test Code 075

No.	Item	Data
1	Unit	ng/dL
2	Decimal places	2
3	Reference low	
4	Reference high	
5	Reschedule low	0.10
6	Reschedule high	8.0
7	Assay range low	0.10
8	Assay range high	8.0
9	Specimen diluent code	
10	Specimen diluent name	
11	Dilution factor for Sp. 1	1
12	Dilution factor for Sp. 2	1
13	Dilution factor for Control	1
14	Default multiplier for DO	1
15	Default multiplier for >H	1
16	Factor A	1.00000e+000
17	Factor B	0.00000e+000
18	Calibration code	5
19	Calibrator replicates	3
20	Calibrator lot	***Enter current cal. lot no.
21	Calibrator Concentration	
22	Cal - 01	0 ng/dL
23	Cal - 02	0.4 ng/dL (example)
24	Cal - 03	1.0 ng/dL (example)
25	Cal - 04	2.5 ng/dL (example)
26	Cal - 05	4.0 ng/dL (example)
27	Cal - 06	9.0 ng/dL (example)
28		
29		
30		
31		
32		
33		
34	Dilution factor for calibrator	1
35	Calibration period	90
36	Incubation time (10/40)	10
37	Assay protocol	1
38	Specimen volume	10
39	Diluent volume	140
40	Conjugate volume	0
41	Diluted conjugate volume	0
42	Pretreatment	0
43	Pretreatment specimen volume	0
44	Pretreatment Reagent code	0
45	Pretreatment Reagent name	0
46	Pretreatment Reagent 1 volume	0
47	Pretreatment Reagent 2 volume	0
48	System Factor	1
49	Calibration Code Check	046
50	Virtual Concentration	0.0
51	Graph Origin	0.0
52	Calculation with Dilution Factor	Yes

Free Thyroxine
ST AIA-PACK FT4

Name and Intended Use

ST AIA-PACK FT4 is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of non-protein-bound (free) thyroxine (FT4) in human serum or heparinized plasma on specific Tosoh AIA System analyzers.

Summary and Explanation of Test

L-thyroxine (3, 5, 3-, 5-L-tetraiodothyronine (T4)) produced by the thyroid gland, circulates in the blood 99.97% bound to plasma proteins including thyroxine-binding globulin (TBG), thyroxine binding prealbumin (TBPA) and albumin.[1,2] Approximately 0.03% of the total circulating thyroxine is unbound. This free T4 (FT4) is believed to be the physiologically active portion of the thyroxine which stimulates the metabolism and controls, via the pituitary, the feedback system involving the release of TSH.[3]

Historically, measurement of total serum T4 (bound + free) has been used to assess the clinical status of the thyroid gland.[4,5] However, this analysis is not diagnostically accurate when significant changes occur in the serum binding proteins.[6,7] Alterations in TBG concentration, pregnancy, oral contraceptives, estrogen therapy or drugs which alter the binding of thyroxine to the carrier proteins may cause corresponding changes in the total T4 when unbound free thyroxine levels remain relatively unchanged.[8,9] Therefore, measurement of the free T4 (FT4) typically correlates more closely to the patient's actual thyroid status than the total.[10]

Principle of the Assay

The ST AIA-PACK FT4 is a competitive enzyme immunoassay which is performed entirely within the AIA-PACK. The thyroxine not bound to serum proteins (free T4) competes with enzyme-labeled T4 for a limited number of binding sites on a T4-specific antibody immobilized on magnetic beads. After incubation, the beads are washed to remove the unbound enzyme-labeled T4 and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled T4 that binds to the beads is inversely proportional to the free T4 concentration in the test sample. A standard curve using a range of known standard concentrations is constructed and unknown sample free T4 concentrations are calculated using this curve.

Material Provided (ST AIA-PACK FT4, Cat. No. 025268)

5 trays x 20 test cups (ST AIA-PACK FT4 Test Cup)

Plastic test cups containing lyophilized magnetic beads with anti-T4 rabbit polyclonal antibody and thyroxine (T4) conjugated to bovine alkaline phosphatase with 0.1% sodium azide as a preservative.

Materials Required But Not Provided

The following materials are not provided but are required to perform Free Thyroxine analysis using the ST AIA-PACK FT4 (Cat. No. 025268) on specific Tosoh AIA Systems. They are available separately from Tosoh.

Materials	Cat. No.
AIA-SYSTEMS:	
AIA-360	019945
AIA-600II	019014
AIA-600II BCR	019328
AIA-900	022930
AIA-900 9tray Sorter	022931
AIA-900 19tray Sorter	022932
AIA-1800 ST	019836
AIA-1800 LA	019837
AIA-2000 ST	022100
AIA-2000 LA	022101
AIA-PACK:	
AIA-PACK Substrate Set II	020968
AIA-PACK Substrate/Reconstituent	
AIA-PACK FT3 Calibrator Set	020368
Calibrator #1 0 ng/dL	
Calibrator #2 0.4 ng/dL (approx.)	
Calibrator #3 1.0 ng/dL (approx.)	
Calibrator #4 2.5 ng/dL (approx.)	
Calibrator #5 4.0 ng/dL (approx.)	
Calibrator #6 9.0 ng/dL (approx.)	
AIA-PACK Wash Concentrate Set	020955
AIA-PACK Diluent Concentrate Set	020956
AIA-PACK Detector Standardization Test Cups	020970
Sample Cups	018581
ADDITIONAL REQUIREMENTS: (Except AIA-360)	
Pipette Tips (1000/Pkg)	019215
Tip Rack (Empty)	019216
Preloaded Pipette Tips (96 Tips X 50 Racks)	996010
Preloaded Pipette Tips (96 Tips X 5 Racks)	996005

Only materials obtained from Tosoh should be used. Materials obtained elsewhere should not be substituted since assay performance is based strictly on Tosoh materials.

Warnings and Precautions

- The ST AIA-PACK FT4 is intended for in vitro diagnostic use only.
- The ST AIA-PACK FT4 contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Human sera is not used in the preparation of this product, however, since human specimens will be used for samples and other quality control products in the lab may be derived from human serum, please use standard laboratory safety procedures in handling all specimens and controls.
- Do not use beyond the expiration date.

Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at the specified temperature.

Materials	Cat. No.
Refrigerator Temperature (2° - 8° C):	
ST AIA-PACK FT4	025268
AIA-PACK FT4 Calibrator Set	020368
AIA-PACK Substrate Set II	020968
AIA-PACK Wash Concentrate	020955
AIA-PACK Diluent Concentrate	020956
Room Temperature (18° - 25° C):	
AIA-PACK Detector Standardization Test Cups	020970

ST AIA-PACK FT4 test cups may be stored for up to 24 hours at a room temperature of 18° - 25° C. Calibrators must be kept tightly sealed and refrigerated at 2° - 8° C. After opening, calibrators should be used within 24 hours. Reconstituted substrate solution is stable for 3 days at 18-25°C or 30 days at 2-8°C. Working diluent and wash solutions are stable for 30 days at room temperature (18° - 25° C). Reagents should not be used if they appear cloudy or discolored.

Specimen Collection and Handling

Serum or heparinized plasma is required for the assay. EDTA and citrated plasma SHOULD NOT BE USED.

No special patient preparation is necessary. When using serum, a venous blood sample is collected aseptically without additives (Red top tube). Store at 18-25°C until a clot has formed (usually 15 - 45 minutes), then centrifuge to obtain the serum specimen for assay. SST or gel tubes have not been validated.

To use heparinized plasma, a venous blood sample is collected aseptically with the designated additive. Centrifuge and separate plasma from the packed cells as soon as possible.

Samples may be stored at 2° - 8° C for up to 24 hours prior to analysis. If the analysis cannot be done within 24 hours, the sample should be stored frozen at -20° C or below for up to 60 days.

Repeated freeze-thaw cycles should be avoided. Turbid serum samples or samples containing particulate matter should be centrifuged prior to testing. Prior to assay, bring frozen samples to room temperature (18° - 25° C) slowly and mix gently.

The sample required for analysis is 10 µL.

Procedure

1) Reagent Preparation

1a) Substrate Solution

Bring all reagents to room temperature (18° - 25° C) before preparing the working reagent. Add the entire contents of the Substrate Reconstituent (100 mL) to the lyophilized Substrate and mix thoroughly to dissolve the solid material.

1b) Wash Solution

Add the entire contents of the Wash Concentrate (100 mL) to approximately 2.0 L of CAP Class I or NCCLS (CLSI) Type I Reagent Grade water, mix well, and adjust the final volume to 2.5 L.

1c) Diluent

Add the entire contents of the Diluent Concentrate (100 mL) to approximately 4.0 L of CAP Class I or NCCLS (CLSI) Type I Reagent Grade water, mix well, and adjust the final volume to 5.0 L.

2) Calibration

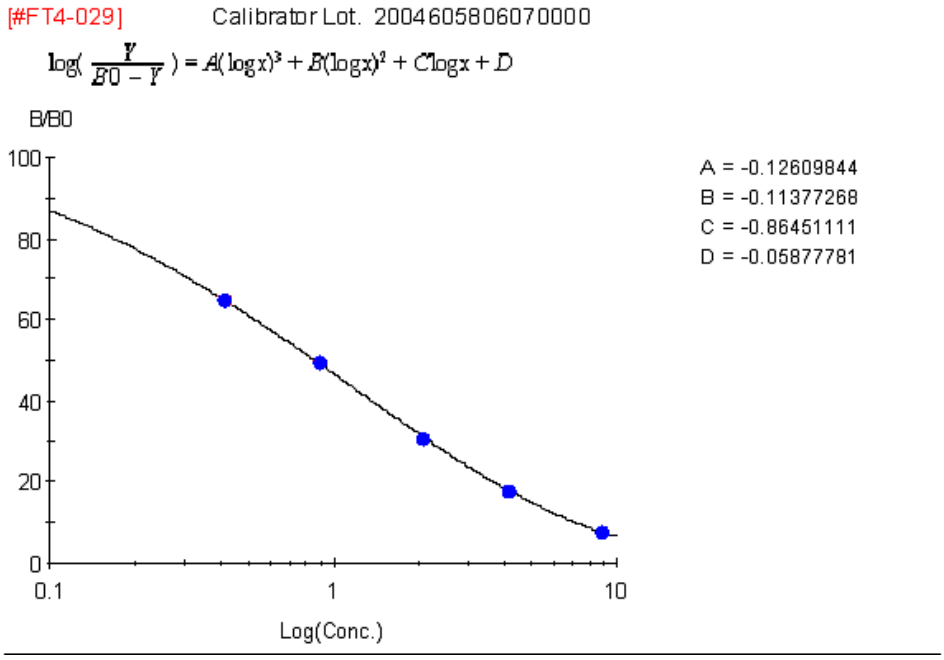
2a) Calibration Curve

The calibrators for use with the ST AIA-PACK FT4 are prepared gravimetrically and are compared to internal reference standards.

The calibration curve for the ST AIA-PACK FT4 is stable for up to 90 days. Calibration stability is monitored by quality control performance and is dependent on proper reagent handling and AIA System maintenance according to the manufacturer's instructions.

Recalibration may be necessary more frequently if controls are out of the established range for this assay or if certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, or detector lamp adjustment or change). For further information regarding instrument operation, consult the AIA System Operator's Manual.

The sample calibration curve below shows the algorithm used to calculate the results. This is an example only. Actual results will vary depending on the Instrument type and lot number used.



2b) Calibration Procedure

i) Refer to the appropriate AIA System Operator's Manual for procedural instructions.

ii) Verify that both the calibrator lot and concentration numbers have been correctly entered into the software.

iii) Calibrators for ST AIA-PACK FT4 are lyophilized and should be reconstituted with 1 mL of CAP Class I or NCCLS (CLSI) Type I Reagent Grade water. Tosoh recommends that all calibrators be run in triplicate.

2c) Calibration Acceptability criteria

i) Since there is an inverse relationship between concentration and rate, the rates should decrease as the concentration increases.

ii) The replicate values should be within a 10% range.

2d) Calibration Review and Acceptance

i) Review the calibration curve carefully, using the criteria listed above.

ii) Edit the calibration if necessary, then accept the calibration.

iii) For further information regarding calibration, consult the specific AIA System Operator's Manual.

3) Quality Control

3a) Commercially Available Controls

Commercially available controls should be run at least once per day. It is recommended that at least two (2) levels of controls, normal and abnormal, be used. Laboratory policy for this particular assay designates the following:

Control Material: _____
Frequency: _____

Lot number of control material, acceptable limits, and corrective action to be taken if controls do not meet laboratory criteria will be found in a separate quality control document maintained by the laboratory.

3b) Quality Control Procedure

i) Assay quality control specimens as instructed in the specific Operator's Manual for your analyzer. In addition, refer to the AIA System Operator's Manual for detailed instructions on defining and editing the files.

ii) Quality control material to be run with this assay is defined by individual laboratory policy.

4) Specimen Processing

4a) Preparation

Following specific instructions in the Operator's Manual for the analyzer, place samples on the instrument appropriately.

4b) Assay Procedure

i) Ensure a sufficient quantity of ST AIA-PACK FT4 test cups for the number of samples to be run.

ii) Load patient samples as instructed in the Operator's Manual and proceed with analysis.

Procedural Notes

1. Lyophilized Substrate must be completely dissolved.
2. Ligand assays performed by the Tosoh AIA Systems require that the laboratory use water designated by the College of American Pathologists as Class I or by NCCLS (CLSI) as Type I. Water should be tested at least once per month and should be free of particulate matter including bacteria. The pH of the water should also be routinely tested. For further information, consult the NCCLS (CLSI) document “Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline – Third Edition”; October 1997, NCCLS Document C3-A3.
3. If a specimen free thyroxine concentration is found to be greater than 8.0 ng/dL, results should be reported as greater than 8 ng/dL. Dilution or recovery studies cannot be performed on the Free Thyroxine assay. The equilibrium between bound and free hormone in the Free Thyroxine assay is altered both by dilutions and the addition of measured amounts of thyroxine.
4. The AIA Systems can store two different calibration curves for each analyte at one time. Therefore, up to two different lots of ST AIA-PACK FT4 test cups can be used during the same run.
5. If the assay specifications for this test are not already in the system software, the specifications must be entered under test code 075.

Calculation of Results

The AIA Systems perform all sample and reagent handling operations automatically. The AIA Systems read the rate of fluorescence produced by the reaction and automatically convert the rate to Free Thyroxine concentration in ng/dL.

Evaluation of Results

Quality Control

In order to monitor and evaluate the precision of the analytical performance, it is recommended that commercially available control samples be assayed daily.

The minimum recommendations for the frequency of running internal control material are:

- After calibration, three levels of controls are run in order to accept the calibration curve.
- The three levels of controls are also repeated after calibration when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe or detector lamp adjustment or change).

After daily maintenance, at least two levels of the control should be run in order to verify the overall performance of the Tosoh AIA System Analyzers.

If one or more control sample value(s) is out of the acceptable range, it will be necessary to investigate the validity of the calibration curve before reporting patient results.

Standard laboratory procedures should be followed in accordance with the regulatory agency under which the laboratory operates.

Limitations of the Procedure

For diagnostic purposes, the results obtained from this assay should be used in conjunction with other data (e.g., symptoms, results of other tests, clinical impressions, therapy, etc.).

Using ST AIA-PACK FT4, the highest concentration of Free Thyroxine measurable is approximately 8.0 ng/dL, and the lowest measurable concentration is 0.10 ng/dL (assay sensitivity).

Although the approximate value of the highest calibrator is 9.0 ng/dL, the exact concentration may be slightly different. The assay specification, Assay Range High, should be defined as the upper limit of the assay range, 8.0 ng/dL.

Although hemolysis has an insignificant effect on the assay, hemolyzed samples may indicate mistreatment of a specimen prior to assay and results should be interpreted with caution.

Lipemia has an insignificant effect on the assay except in the case of gross lipemia where spatial interference may occur.

Circulating autoantibodies to T4 may interfere with the free thyroxine measurements.

Hormone-binding inhibitors may interfere with the free T4 assay. Heparin may cause in vivo effects in free T4 assays. Blood collection for free T4 assays should not be performed concurrent with administration of heparin therapy.

Certain medications may interfere with assay performance. All results should be interpreted with respect to the clinical picture of the patient.

Drugs which affect the binding of T4 to the thyroid hormone carrier proteins or affect the metabolism of T4 to T3 may complicate the interpretation of free thyroxine results. In patients with severe non-thyroidal illness (NTI) in whom the free T4 yields results indicating hypothyroidism, it is suggested that the AIA-PACK TSH 3rd-Gen or ST AIA-PACK TSH assay be run to confirm this diagnosis.

For a more complete understanding of the limitations of this procedure, please refer to the Specimen Collection and Handling, Warnings and Precautions, Storage and Stability, and Procedural Notes sections in this insert sheet.

Expected Values

Each laboratory should determine a reference interval which corresponds to the characteristics of the population being tested. As with all diagnostic procedures, clinical results must be interpreted with regard to concomitant medications administered to the patient.[11]

Reference Ranges

The interval given here was determined in serum samples from 103 apparently healthy individuals.

Reference Interval	=	0.75 - 1.54 ng/dL	(9.68 - 19.9 pmol/L)

Conversion Factors

FT4 concentrations in this application are in units of ng/dL. Conversion to SI units of pmol/L may be made using the following equation:

pmol FT4/L = ng FT4/dL x 12.9

Performance Characteristics

2) Precision

2a) Intra-assay precision

The intra-assay (within run) precision was determined using three controls in a total of 20 runs. Within each run, one set of duplicates per control was assayed. The mean of each duplicate was used to obtain the pooled standard deviation (SD), which was then used to calculate the coefficient of variation.

Sample	Mean (ng/dL)	Pooled SD (ng/dL)	Coefficient of Variation (%)
Sample A	0.55	0.018	3.3
Sample B	1.62	0.043	2.7
Sample C	4.27	0.076	1.8

2b) Inter-assay precision

The inter-assay (between run) precision coefficient of variation was evaluated at three different concentrations by analyzing samples in triplicate in 20 separate runs.

Sample	Number of Replicates	Mean (ng/dL)	Standard Deviation (ng/dL)	Coefficient of Variation (%)
Sample A	20	0.56	0.02	4.0
Sample B	20	1.634	0.04	2.4
Sample C	20	4.27	0.09	2.2

2c) Total precision

Total precision was determined by the duplicate assay of three controls in 20 separate runs. The means of each run were used to calculate the standard deviation (SD) and coefficient of variation (CV).

Sample	Mean (ng/dL)	Standard Deviation (ng/dL)	Coefficient of Variation (%)
Sample A	0.55	0.027	4.9
Sample B	1.2	0.056	3.5
Sample C	4.27	0.101	2.4

Specificity

The following substances were tested for cross-reactivity. The cross-reactivity (%) is the percent of the compound which will be identified as FT4. If these compounds are present in the specimen at the same concentration as FT4, the final result will be increased by these percentages.

Compound	Cross-reactivity (%)
L-Thyroxine (L-T4)	100.0
D-Thyroxine (D-T4)	29.5
L-Triiodothyronine (L-T3)	1.1
D-Triiodothyronine (D-T3)	0.6
3, 3, 5-Triiodothyropropionic acid	1.8
3, 5-Diiodothyropropionic acid	0.13
3, 5-Diiodo-L-tyrosine	<0.1

Sensitivity

The minimal detectable concentration (MDC) of Free Thyroxine is estimated to be 0.10 ng/dL. The MDC is defined as that concentration of FT4 which corresponds to the rate of fluorescence that is two standard deviations from the mean rate of fluorescence of 20 replicate determinations of a zero calibrator.

Interference

Interference is defined, for purposes of this study, to be recovery outside of 10% of the known specimen mean concentration.

- Added hemoglobin (up to 390 mg/dL), free bilirubin (up to 17 mg/dL) and conjugated bilirubin (up to 19 mg/dL) do not interfere with the assay.
- Lipemia, as indicated by added triglyceride (up to 1660 mg/dL), does not interfere with the assay.
- Added TBG up to 50 μg/mL, human serum albumin up to 4 g/dL, and prealbumin up to 500 μg/mL do not interfere with the assay.
- Sodium oleate, used to test the effect of NEFA, does not interfere with the assay when it is added in concentrations up to 1.25 mM.

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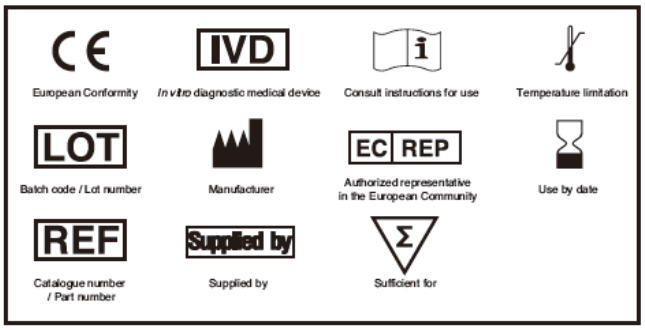

AIA-PACK
FT4 Calibrator Set

Intended Use

The AIA-PACK FT4 Calibrator Set is intended for IN VITRO DIAGNOSTIC USE ONLY for the calibration the ST AIA-PACK FT4 Assay.

Summary and Explanation

The AIA-PACK FT4 Calibrator Set contains human serum with assigned levels of non-protein bound (free) thyroxine (FT4). Calibration should be performed according to the schedule indicated in the AIA System Operator's Manual. The calibrators for use with the AIA-PACK FT4 are prepared gravimetrically and are compared to internal reference standards.

Materials Provided (Cat. No. 020368)

2 x 1 mL	Calibrator	No. 1	0.0 ng/dL
			Human serum containing no detectable concentration of FT4 (0 ng FT4/dL), and 0.1% sodium azide as a preservative. (Lyophilized)
2 x 1 mL	Calibrator	No. 2	0.4 ng/dL (approx.)
		No. 3	1.0 ng/dL (approx.)
		No. 4	2.5 ng/dL (approx.)
		No. 5	4.0 ng/dL (approx.)
		No. 6	9.0 ng/dL (approx.)
			Human serum containing the assigned concentration of FT4 (described on each vial), and 0.1% sodium azide as a preservative. (Lyophilized)

Warnings and Precautions

- The AIA-PACK FT4 Calibrator Set is for in vitro diagnostic use.
- These materials contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Human sera used in the preparation of this product has been tested by FDA cleared methods and found negative for the presence of HBsAg and antibody to HIV-1 and HCV. Because no test method can offer complete assurance that products derived from human blood will not transmit infectious agents, it is recommended that this product be handled with the same precautions as used for patient samples.
- Do not use beyond the expiration date.

Preparation and Storage

- Using volumetric pipettes, reconstitute the lyophilized calibrators accurately to the volume of 1 mL with distilled water. Allow the lyophilized material to fully dissolve, then mix the calibrators gently but thoroughly prior to performing the calibration.
- Bring calibrator to room temperature (18° - 25° C) for use.
- Always store the Calibrator Set in an upright position at 2° - 8° C when not in use.

Stability

When stored unopened and refrigerated at 2° - 8° C, the AIA-PACK FT4 Calibrator Set is stable until the expiration date on the label. After opening, the calibrators should be used within 24 hours.

Procedure

Refer to the CALIBRATION PROCEDURE in the AIA-PACK section of this analyte application. For additional procedural instructions regarding calibration, refer to the AIA System Operator's Manual.

Calibration

1. Verify that both the calibrator lot and concentrations have been correctly entered into the analyzer software.
2. Add the appropriate amounts of each calibrator to sample cups (refer to the instrument worklist for the amount required in each sample cup).
3. Place the sample cups and the test cups on the analyzer as indicated on the worklist.
4. Tosoh recommends that all calibrators be run in triplicate.

Assignment of Values

The AIA-PACK FT4 Calibrator Set contains assigned concentrations of Free Thyroxine. The assigned value is determined on a lot-to-lot basis and is designed to provide an assay calibration range of approximately 0 to 8.0 ng/dL of Free Thyroxine. The calibrators in this set are prepared gravimetrically and are compared to internal reference standards.

Results

1) Since there is an inverse relationship between concentration and rate, the rates should decrease as the concentration increases.
2) The replicate values should be within a 10% range.

Limitations

The AIA-PACK FT4 Calibrator Set is designed solely for use with AIA-PACK assay procedures.

References

1. AIA-System Analyte Application Manual. Tosoh Bioscience, Inc., South San Francisco, CA.
2. AIA-Analyzer Operator's Manual. Tosoh Bioscience, Inc., South San Francisco, CA.

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