AIA-360 Assay Specifications
ST INTACT PTH Test Code 052

No.	Item	Data
1	Calib. Req	Show
2	Cal 1	0 pg/mL
3	Cal 2	15 pg/mL (example)
4	Cal 3	50 pg/mL (example)
5	Cal 4	200 pg/mL (example)
6	Cal 5	800 pg/mL (example)
7	Cal 6	2400 pg/mL (example)
8	Cal Lot L	
9	Cal Lot R	
10	Unit	pg/ml
11	Smpl. Vol	75
12	Dil. Vol	50
13	Assay L	1.0
14	Assay H	2000
15	Ref. L	
16	Ref. H	
17	Decimal	1

Visible only in Test Mode

18	Test Name	#PTH
19	Calib. No	6
20	Calib. Mul	3
21	Calib. Equ	3
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 INTACT PTH Test Code 052

Screen	Item	Data
Screen 1		
	Lot	***Enter current cal. lot no.
	Cal 1	0 pg/mL
	Cal 2	15 pg/mL (example)
	Cal 3	50 pg/mL (example)
	Cal 4	200 pg/mL (example)
	Cal 5	800 pg/mL (example)
	Cal 6	2400 pg/mL (example)
Screen 2		
	Name	#PTH
	Unit	pg/mL
	Smpl	75
	Dil	50
	2Reag	0
	Code	0
	Assay Range Low	1.0
	Assay Range High	2000
	Reference Range Low	
	Reference Range High	
	DP (No. of decimal points)	1
Screen 3		
	Code	52
	No. (Calibrators)	6
	Mul. (Replicates)	3
	Equ	3
	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)	52
	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 INTACT PTH Test Code 052

No.	Item	Data
1	Code	52
2	ACT	0
3	Analyte	#PTH
4	Lot	***Enter current cal lot no.
5	CAL 1	0 pg/mL
6	CAL 2	15 pg/mL (example)
7	CAL 3	50 pg/mL (example)
8	CAL 4	200 pg/mL (example)
9	CAL 5	800 pg/mL (example)
10	CAL 6	2400 pg/mL (example)
11	Cal lot L	
12	Cal lot R	
13	Unit	pg/mL
14	Decimal	1
15	Assay Low	1
16	Assay High	2000
17	Reference Low	
18	Reference High	
19	Reschedule Low	1
20	Reschedule High	2000
21	Factor A	1
22	Factor B	0
23	Sample Volume	75
24	Diluent Volume	50
25	2 Step reagent dispensing volume	0
26	Calibration code	52
27	CAL. No.	6
28	CAL. MUL.	3
29	CAL. EQU.	3
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	10
36	DIL. AH.	5
37	DIL. CALC.	1
38	DIL. CODE	52
39	DIL. NAME	#PTH
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 INTACT PTH Test Code 052

No.	Item	Data
1	Unit	pg/mL
2	Decimal places	1
3	Reference low	
4	Reference high	
5	Reschedule low	1.0
6	Reschedule high	2000
7	Assay range low	1.0
8	Assay range high	2000
9	Specimen diluent code	052
10	Specimen diluent name	#PTH
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	10
15	Default multiplier for >H	5
16	Factor A	1.00000e+000
17	Factor B	0.00000e+000
18	Calibration code	003
19	Calibrator replicates	3
20	Calibrator lot	***Enter current cal. lot no.
21	Calibrator Concentration	
22	Cal - 01	0 pg/mL
23	Cal - 02	15 pg/mL (example)
24	Cal - 03	50 pg/mL (example)
25	Cal - 04	200 pg/mL (example)
26	Cal - 05	800 pg/mL (example)
27	Cal - 06	2400 pg/mL (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	75
39	Diluent volume	50
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	1.00
49	Calibration Code Check	052
50	Virtual Concentration	0.0
51	Graph Origin	0.0
52	Calculation with Dilution Factor	Yes

AIA-2000 Assay Specifications
ST INTACT PTH Test Code 052

No.	Item	Data
1	Unit	pg/mL
2	Decimal places	1
3	Reference low	
4	Reference high	
5	Reschedule low	1.0
6	Reschedule high	2000
7	Assay range low	1.0
8	Assay range high	2000
9	Specimen diluent code	052
10	Specimen diluent name	#PTH
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	10
15	Default multiplier for >H	5
16	Factor A	1.00000e+000
17	Factor B	0.00000e+000
18	Calibration code	003
19	Calibrator replicates	3
20	Calibrator lot	***Enter current cal. lot no.
21	Calibrator Concentration	
22	Cal - 01	0 pg/mL
23	Cal - 02	15 pg/mL (example)
24	Cal - 03	50 pg/mL (example)
25	Cal - 04	200 pg/mL (example)
26	Cal - 05	800 pg/mL (example)
27	Cal - 06	2400 pg/mL (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	75
39	Diluent volume	50
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	1.00
49	Calibration Code Check	052
50	Virtual Concentration	0.0
51	Graph Origin	0.0
52	Calculation with Dilution Factor	Yes

Intact Parathyroid Hormone
ST AIA-PACK Intact PTH

Name and Intended Use

ST AIA-PACK Intact PTH is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of the levels of parathyroid hormone in human serum and EDTA plasma on specific Tosoh AIA System analyzers. Measurements of parathyroid hormone levels are used in the differential diagnosis of hypercalcemia (abnormally high levels of calcium in the blood) and hypocalcemia (abnormally low levels of calcium in the blood) resulting from disorders of calcium metabolism.

Summary and Explanation of Test

Parathyroid hormone (PTH) is a polypeptide of 84 amino acids (MW 9800 Daltons). PTH raises blood ionized calcium levels by promoting bone dissolution and releasing calcium phosphate from the bone to the extra cellular fluid through direct action on bone. PTH also promotes reabsorption of ionized calcium and depresses the renal reabsorption of phosphate through action on the renal tubular. Hence, PTH is an important hormone to optimize calcium ion concentrations in the blood [1-3]. PTH is rapidly metabolized in the liver and kidney primarily to biologically active N-terminal and biologically inactive C-terminal fragments with much longer half-lives. C-terminal fragments, excreted from the kidney, are grossly elevated in subjects with renal insufficiency [4-5]. Intact PTH assays accurately reflects PTH-secretion kinetics due to less influence by declining renal function. [6-9]

Therefore, Intact PTH is an important marker to diagnose the diseases of the parathyroid gland and the kidney. For example in hyperparathyroidism and secondary hyperparathyroidism PTH is used to judge success or failure of surgery in the removal of malignant parathyroid gland, and to monitor postoperative recovery.

Principle of the Assay

The ST AIA-PACK Intact PTH is a two-site immunoenzymometric assay which is performed entirely in the AIA-PACK. Intact PTH present in the test sample is bound with polyclonal antibody immobilized on magnetic beads and enzyme-labeled polyclonal antibody in the AIA- PACK. The magnetic beads are washed to remove unbound enzyme-labeled polyclonal antibody and are then incubated with a fluorogenic substrate 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled polyclonal antibody that binds to the beads is directly proportional to Intact PTH concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve.

Material Provided (ST AIA-PACK Intact PTH, Cat. No. 025213)

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

Plastic test cups containing lyophilized twelve magnetic beads coated with anti-PTH goat polyclonal antibody and anti-PTH goat polyclonal antibodies 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 Intact Parathyroid Hormone analysis using the ST AIA-PACK Intact PTH (Cat. No. 025213) 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 Intact PTH Calibrator Set	020313
Calibrator #1 0 pg/mL	
Calibrator #2 15 pg/mL (approx.)	
Calibrator #3 50 pg/mL (approx.)	
Calibrator #4 200 pg/mL (approx.)	
Calibrator #5 800 pg/mL (approx.)	
Calibrator #6 2400 pg/mL (approx.)	
AIA-PACK Intact PTH Sample Diluting Solution	025513
AIA-PACK Intact PTH Control Set	025413
AIA-PACK Wash Concentrate Set	020955
AIA-PACK Diluent Concentrate Set	020956
AIA-PACK Detector Standardization Test Cups	020970
AIA-PACK Sample Treatment Cups	020971
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 Intact PTH is intended for in vitro diagnostic use only.
- Test cups from different lots should not be mixed within a tray.
- The ST AIA-PACK Intact PTH 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 serum 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 handlings all specimens and controls.
- Do not use beyond the expiration date.
- The ST AIA-PACK Intact PTH has been designed so that the high dose “hook effect” is not a problem for the vast majority of samples. Samples with Intact PTH concentrations between 2,000 and 100,000 pg/mL will read > 2,000 pg/mL. The “hook effect” phenomenon may occur at Intact PTH concentrations > 100,000 pg/mL.

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 Intact PTH	025213
AIA-PACK Intact PTH Calibrator Set	025313
AIA-PACK Intact PTH Sample Diluting Solution	025513
AIA-PACK Intact PTH Control Set	025413
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
AIA-PACK Sample Treatment Cups	020971

ST AIA-PACK Intact PTH test cups may be stored for up to 24 hours at 18° - 25° C. Calibrators must be kept tightly sealed and refrigerated at 2° - 8° C. After opening, calibrators are best recommended to use once and for all within 24 hours. After opening, SAMPLE DILUTING SOLUTION should be used within 7 days. They should be used after equilibrating to room temperature (15 - 25° C) for about 30 minutes. The Sample Diluting Solution can be used for up to 90 days provided that 1) it is used for manual dilutions ONLY, and 2) the vials are kept tightly sealed and refrigerated immediately after use. 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 18° - 25° C. Reagents should not be used if they appear cloudy or discolored.

Specimen Collection and Handling

Serum, EDTA plasma is required for the assay. Heparinized or Citrated plasma **SHOULD NOT BE USED**.

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.

If using EDTA plasma, a venous blood sample is collected aseptically with designated additive (10-11). Centrifuge and separate plasma from the packed cells as soon as possible.

Specimen types should not be used interchangeably during the serial monitoring of an individual patient. Measured concentrations may vary slightly between sample types in certain patients.

Specimen 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 18-25°C slowly and mix gently.

The sample required for analysis is 75 µL.

Procedure

1) Reagent Preparation

1a) Substrate Solution

Bring all reagents to 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

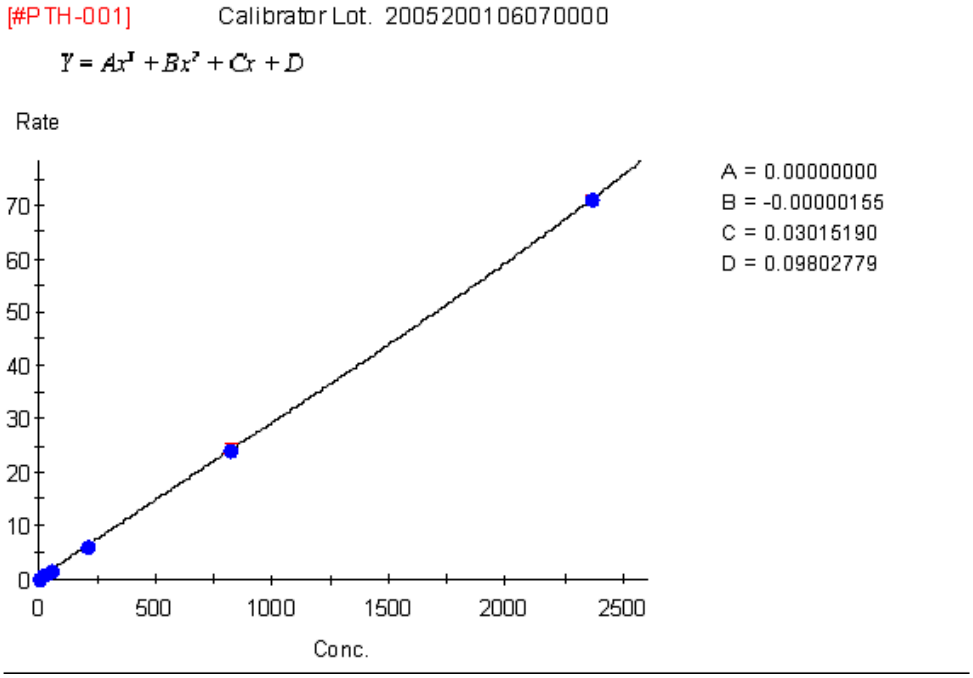
2a) Calibration Curve

The calibrators for use with the ST AIA-PACK Intact PTH are prepared gravimetrically.

The calibration curve for the ST AIA-PACK Intact PTH 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) Calibrator (1) for ST AIA-PACK Intact PTH is provided ready to use. Tosoh recommends that all calibrators be run in triplicate.

iv) Calibrators (2)-(6) for ST AIA-PACK Intact PTH are lyophilized. All levels should be reconstituted with 1mL 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) The mean rate for the zero calibrator should be < 0.5 nM/sec.

ii) Since there is a direct relationship between concentration and rate, the rates should increase as the concentration increases.

iii) 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 AIA-2000, AIA-1800 and the AIA Nex·IA, controls may be defined to validate the calibration prior to acceptance. 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 Intact PTH 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. Note: The AIA-900, AIA-600II, AIA-1800 and AIA-2000 will require AIA-PACK Sample Treatment Cups if onboard dilutions are utilized.

Procedural Notes

1) Lyophilized Substrate must be completely dissolved.

2) If a specimen Intact PTH concentration is found to contain greater than the linearity limit of the assay, 2000 pg/mL; the specimen should be diluted with the AIA-PACK Intact PTH Sample Diluting Solution and re-assayed according to the Assay Procedure. The recommended dilution for specimen containing greater than 2,000 pg/mL is 1:10 or 1:100. The dilution factor should be entered into the software. For further information on the dilution of specimens, refer to the AIA System Operator's Manual.

3) 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 Intact PTH test cups can be used during the same run.

4) If the assay specifications for this test are not already in the system software, the specifications must be entered under test code 052.

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 Intact Parathyroid Hormone concentration in pg/mL.

For samples requiring dilution, AIA-2000, AIA-1800, AIA-600 II and the AIA Nex·IA will automatically perform dilutions and calculate results if the dilution factors are entered into the software. For detailed information regarding programming dilutions, consult the appropriate Operator's Manual.

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, two levels of controls are run in order to accept the calibration curve.
- The two 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 Intact PTH, the highest concentration of Intact Parathyroid Hormone measurable without dilution is approximately 2,000 pg/mL, and the lowest measurable concentration is 1.0 pg/mL (assay sensitivity).

The exact linearity of the ST AIA-PACK Intact PTH depends on the particular lot of calibrator in use. Although the approximate value of the highest calibrator is 2,200 pg/mL, the exact concentration may be slightly different. The assay specification, Assay Range High, should be defined as the upper limit of the assay range, 2000 pg/mL.

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.

Specimens from patients who have received preparations of goat polyclonal antibodies for diagnosis or therapy may contain human anti-goat antibodies. Such specimens may show falsely elevated values when tested for Intact Parathyroid Hormone.

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

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.

Reference Ranges

The interval given here was determined in 144 EDTA plasma specimens of apparently healthy adults. The 144 specimens were measured using the AIA-1800 instrument.

Mean = 45.9

SD = 18.8

Mean + 2SD = 83.5

Mean – 2SD = 8.2

Reference interval = 8.2 – 83.5 pg/mL

Sample Range: 9.5 - 98 pg/ml

Performance Characteristics

1) Accuracy

1a) Recovery:

Three serum samples and three EDTA plasma samples were spiked with three different levels of Intact PTH and assayed before and after spiking.

Sample	Initial Value (pg/mL)	Intact PTH Added (pg/mL)	Expected Value (pg/mL)	Measured Value (pg/mL)	Percent Recovery (%)
Serum A1	20.1	94.5	114.6	114.9	100.3
	20.1	47.3	67.4	69.4	103.0
	20.1	23.6	43.7	44.0	100.6
Serum B1	11.3	94.5	105.8	115.4	109.0
	11.3	47.3	58.6	60.7	103.6
	11.3	23.6	34.9	34.3	98.1
Serum C1	22.5	94.5	117.0	126.9	108.5
	22.5	47.3	69.8	75.2	107.8
	22.5	23.6	46.1	46.0	99.8
Plasma A1	11.2	94.5	105.7	115.0	108.8
	11.2	47.3	58.4	61.9	106.0
	11.2	23.6	34.8	36.3	104.4
Plasma B1	28.8	94.5	123.4	133.8	108.4
	28.8	47.3	76.1	79.4	104.3
	28.8	23.6	52.5	55.8	106.4
Plasma C1	38.9	94.5	133.4	139.0	104.2
	38.9	47.3	86.1	91.1	105.7
	38.9	23.6	62.5	62.7	100.4

1b) Dilution:

Three serum samples and three EDTA plasma samples containing high concentrations of Intact PTH were serially diluted with AIA-PACK Intact PTH Sample Diluting Solution and assayed.

Sample	Dilution Factor	Expected Value (pg/mL)	Measured Value (pg/mL)	Percent Recovery (%)
Serum A2	none		1278.7	
	7.5/10	959.0	943.4	98.4
	5.0/10	639.3	653.4	102.2
	2.5/10	319.7	320.1	100.1
	1.0/10	127.9	123.3	96.7
Serum B2	none		1356.2	
	7.5/10	1017.2	1042.0	102.4
	5.0/10	678.1	691.1	101.9
	2.5/10	339.1	344.4	101.6
	1.0/10	135.6	132.6	97.8
Serum C2	none		1269.2	
	7.5/10	951.9	989.7	104.0
	5.0/10	634.6	638.8	100.7
	2.5/10	317.3	323.8	102.0
	1.0/10	126.9	127.6	100.6
Plasma A2	none		1263.0	
	7.5/10	947.3	936.1	98.8
	5.0/10	631.5	673.8	106.7
	2.5/10	315.8	319.2	101.1
	1.0/10	126.3	128.5	101.8
Plasma B2	none		1484.3	
	7.5/10	1113.2	1037.2	93.2
	5.0/10	742.2	674.0	90.8
	2.5/10	371.1	339.4	91.5
	1.0/10	148.4	140.0	94.3
Plasma C2	none		1388.0	
	7.5/10	1041.0	1020.1	98.0
	5.0/10	694.0	661.5	95.3
	2.5/10	347.0	347.7	100.2
	1.0/10	138.8	136.7	98.5

1c) Linearity:

The linearity for AIA-Intact PTH was measured on the AIA-1800 Instrument, has been demonstrated to be linear from 1 pg/ml to 2000 pg/ml, within +/- 10% difference in this interval.

This interval was determined using linear regression analysis.

Cal	Concentration (pg/ml)	Dilution Scheme	Expected Value	Measured Value						Ratio of Values Assigned/Obtained
				Replicate 1	Replicate 2	Replicate 3	AVG	SD	CV	
1	0	Undiluted	0	<L	0.43	<L	0.43			0.00
		250µL CAL 2 : 250µL CAL 1	7.9	8.27	8.19	8.38	8.28	0.10	1.2	0.95
2	15.8	Undiluted	15.8	15.34	15.95	16.39	15.89	0.53	3.3	0.99
		250µL CAL 3 : 250µL CAL 2	33.9	34.03	33.42	32.25	33.23	0.90	2.7	1.02
3	52.0	Undiluted	52.0	52.22	51.93	54.05	52.73	1.15	2.2	0.99
		250µL CAL 4 : 250µL CAL 3	129	122.95	123.56	122.43	122.99	0.58	0.5	1.04
4	205	Undiluted	205	217.16	216.46	209.55	214.39	4.21	2.0	0.96
		250µL CAL 5 : 250µL CAL 4	512	493.57	486.18	511.48	497.08	13.01	2.6	1.03
5	819	Undiluted	819	796.65	788.71	793.51	792.96	4.00	0.5	1.03
		100µL CAL 6 : 300µL CAL 5	1207	1079.48	1142.27	1120.22	1113.99	31.86	2.9	1.08
		250µL CAL 6 : 250µL CAL 5	1595	1464.32	1498.66	1412.39	1458.46	43.43	3.0	1.09
		300µL CAL 6 : 100µL CAL 5	1983	1821.22	1840.45	1879.52	1847.06	29.71	1.6	1.07
6	2370	Undiluted	2370	2057.93	2321.22	2287.99	2222.38	143.38	6.5	1.07

2) Precision

2a) Intra-assay precision

Within run precision was determined using six 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 (CV).

Sample	Mean (pg/mL)	Pooled SD (pg/mL)	Coefficient of Variation (%)
Serum A3	27.7	0.892	3.2
Serum B3	243.6	8.683	3.6
Serum C3	1123.5	45.493	4.0
Plasma A3	27.1	1.068	3.9
Plasma B3	247.1	9.517	3.9
Plasma C3	1217.2	40.126	3.3

2b) 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 (pg/mL)	Standard Deviation (pg/mL)	Coefficient of Variation (%)
Serum A3	27.7	1.005	3.6
Serum B3	243.6	8.936	3.7
Serum C3	1123.5	44.540	4.0
Plasma A3	27.1	1.320	4.9
Plasma B3	247.1	11.497	4.7
Plasma C3	1217.2	44.159	3.6

2c) Correlation

A comparison of the Tosoh AIA-PACK PTH (y) with a commericially available PTH test (x) using clinical samples gave the following correlations (pg/ml):

Number of samples measured: 153

y= 1.013x – 10.457

r= 0.997

Specificity

The cross-reactivity of PTH fragments

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

PTH fragment	Cross-reactivity (%)
1-84	100
7-84	107.4
1-34	< 0.02
13-34	< 0.02
39-84	< 0.02
53-84	< 0.02

Limit of Detection

The Limit of detection for the AIA-Intact Parathyroid Hormone is approximately 1.0 pg/mL. The Limit of Detection was determined by assaying 5 replicates of non-zero calibrator and 20 replicates of the sample diluting solution or zero calibrator. Limit of Detection was calculated as follows:

$$\text{Limit of Detection} = \frac{\text{(Assigned value of non-zero calibrator) X 2SD}}{\text{(Mean rate of non-zero cal – mean rate of zero cal)}}$$

$$\text{Limit of Detection} = \frac{52 \text{ X } 0.03}{(1.86\text{-}0.25)} = 0.96$$

Interference

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

- Hemoglobin (up to 440 mg/dL), free bilirubin (up to 17 mg/dL) and conjugated bilirubin (up to 17 mg/dL) do not interfere with the assay.
- Lipemia, as indicated by triglyceride concentrations (up to 1,600 mg/dL), does not interfere with the assay.
- Ascorbic acid (up to 20 mg/dL) does not interfere with the assay.
- Protein, as indicated by human albumin concentrations (up to 5.0 g/dL), does not interfere with the assay.
- Heparin (up to 100 U/mL) does not interfere with the assay.

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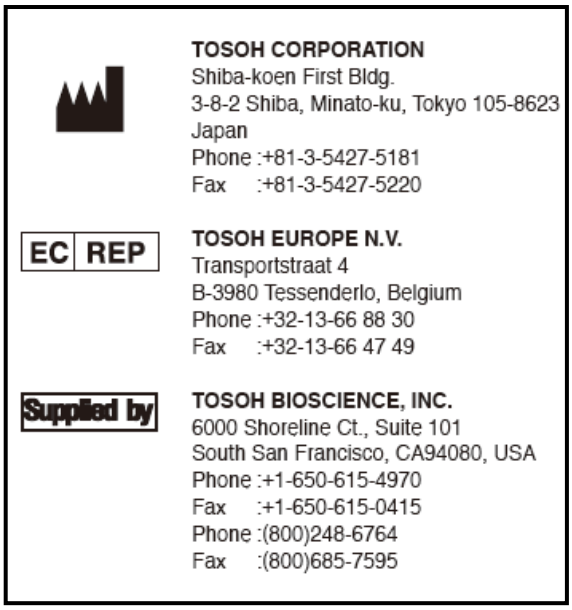
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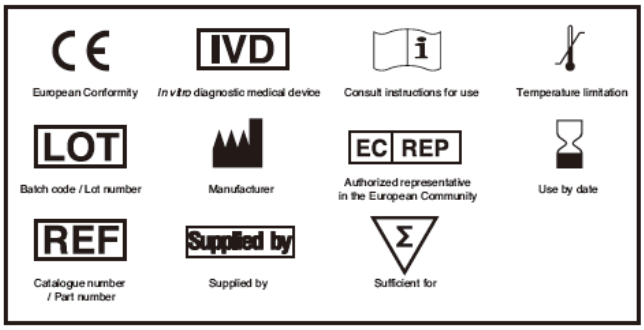
IVD
European Conformity
In vitro diagnostic medical device
Consult instructions for use
Temperature limitation
LOT
Batch code / Lot number
Manufacturer
EC REP
Authorized representative in the European Community
Use by date
REF
Catalogue number / Part number
Supplied by
Σ
Sufficient for

ST AIA-PACK
Intact PTH CALIBRATOR SET

Intended Use

The ST AIA-PACK Intact PTH CALIBRATOR SET is intended for IN VITRO DIAGNOSTIC USE ONLY for the calibration of the ST AIA-PACK Intact PTH Assay.

Summary and Explanation

The ST AIA-PACK Intact PTH CALIBRATOR SET contains a bovine protein matrix with assigned levels of Intact Parathyroid Hormone. Calibration should be performed according to the schedule indicated in the AIA System Operator's Manual. The calibrators in this set have been prepared using an internal primary reference material. The primary reference material was prepared using synthesized Intact PTH.

Material Provided (Cat. No.025313)

2 x 1 mL Calibrator No. 1 0.0 pg/mL

A bovine protein matrix containing no detectable concentration of Intact Parathyroid Hormone (0 pg Intact PTH/mL), and 0.1% sodium azide as preservative.

2 x 1 mL	Calibrator	No. 2	15	pg/mL	(approx.)
		No. 3	50	pg/mL	(approx.)
		No. 4	200	pg/mL	(approx.)
		No. 5	800	pg/mL	(approx.)
		No. 6	2,200	pg/mL	(approx.)

A bovine protein matrix containing the assigned concentration of Intact PTH (described on each vial) and 0.1% proclin as preservative.

Warnings and Precautions

- The ST AIA-PACK Intact PTH CALIBRATOR SET is for in vitro diagnostic use.
- The Calibrator No. 1 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.
- Although material derived from human blood is not used for Intact PTH calibrators, 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 18^{o} - 25^{o} 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 Intact PTH 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 ST AIA-PACK Intact PTH CALIBRATOR SET contains assigned concentrations of Intact Parathyroid Hormone. The assigned value is determined on a lot-to-lot basis and is designed to provide an assay calibration range of approximately 0 to 2,200 pg/mL of Intact Parathyroid Hormone.

Results

- The mean rate for the zero calibrator should be < 0.5 nM/sec.
- Since there is a direct relationship between concentration and rate, the rates should increase as the concentration increases.
- The replicate values should be within a 10% range.

Limitations

The ST AIA-PACK Intact PTH 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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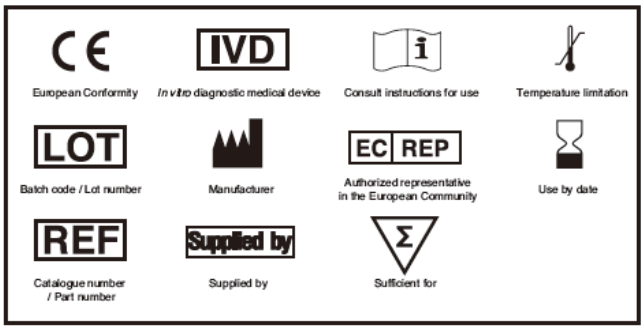


ST AIA-PACK
Intact PTH SAMPLE DILUTING SOLUTION

Intended Use

The ST AIA-PACK Intact PTH SAMPLE DILUTING SOLUTION is intended for IN VITRO DIAGNOSTIC USE ONLY to dilute patient samples that have concentrations of Intact Parathyroid Hormone above the linear range of the assay.

Summary and Explanation

The ST AIA-PACK Intact PTH SAMPLE DILUTING SOLUTION contains a bovine protein matrix with no detectable concentration of Intact Parathyroid Hormone. This Sample Diluting Solution is to be used only with samples that are being tested for Intact Parathyroid Hormone concentrations using the ST AIA-PACK Intact PTH assay.

Materials Provided (Cat. No. 025513)

4 x 4mL	Sample Diluting Solution A bovine protein matrix containing no detectable concentration of Intact Parathyroid Hormone (0 pg Intact PTH/mL), and 0.1% sodium azide as a preservative.

Warnings and Precautions

- The ST AIA-PACK Intact PTH SAMPLE DILUTING SOLUTION 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.
- Although material derived from human blood is not used for Intact PTH Sample Diluting Solution, 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

- The ST AIA-PACK Intact PTH SAMPLE DILUTING SOLUTION is provided ready to use.
- Always store the Sample Diluting Solution in an upright position at 2° - 8° C when not in use.

Stability

When stored unopened and refrigerated at 2 - 8°C, the ST AIA-PACK Intact PTH SAMPLE DILUTING SOLUTION is stable until the expiration date on the label. Sample Diluting Solutions should be used within 7 days of opening, provided the vials are kept tightly sealed and refrigerated at 2 - 8°C.

They should be used after equilibrating to room temperature (15 - 25°C) for about 30 minutes. The Sample Diluting Solution can be used for up to 90 days provided that 1) it is used for manual dilutions ONLY, and 2) the vials are kept tightly sealed and refrigerated immediately after use.

Procedure

Refer to the AIA System Operator's Manual for additional procedural instructions regarding sample dilution.

- If a specimen is found to contain greater than the linearity limit of approximately 2,000 pg/mL, the specimen should be diluted with the Sample Diluting Solution and assayed according to the Procedure in the AIA-PACK section of the analyte application.
- AIA-2000, AIA-1800, AIA-600 II and the AIA Nex·IA will perform dilutions automatically if the dilution factors are entered into the software prior to assaying the diluted sample.
- The recommended dilution for specimen containing greater than the concentration of the highest calibrator is 1:10 or 1:100. However, it is desirable to dilute the samples that contain more than 2,000 pg Intact PTH/mL so that the diluted sample reads between 2,000 and 100,000 pg Intact PTH /mL.

Results

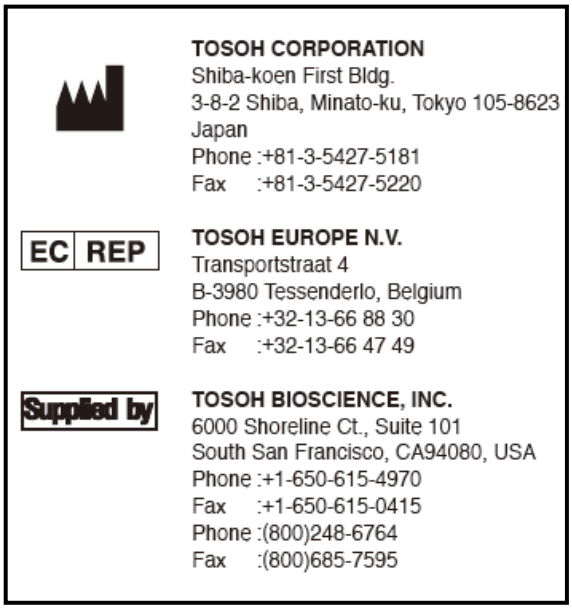
When an auto-dilution is performed, AIA instrument will calculate the final result.

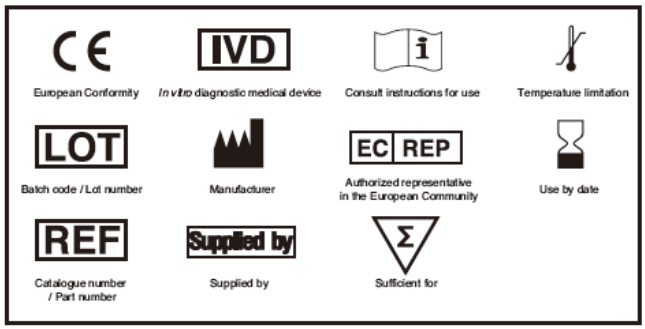
Limitations

The ST AIA-PACK Intact PTH SAMPLE DILUTING SOLUTION 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 Biioscience, Inc., South San Francisco, CA.





AIA-PACK
Intact PTH CONTROL SET

Intended Use

The AIA-PACK Intact PTH CONTROL SET is intended for IN VITRO DIAGNOSTIC USE ONLY for performing quality control procedures with the ST AIA-PACK Intact PTH Assay.

Summary and Explanation

The AIA-PACK Intact PTH CONTROL SET contains buffered bovine serum albumin with the assigned levels of Intact PTH. The Control set has been prepared using the synthesized Intact PTH.

After calibration, controls are run in order to confirm the calibration curve.

Controls are repeated if certain procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe or detector lamp adjustment or change).

After the daily maintenance, at least one level of the control is run in order to verify the overall performance of the AIA system.

Materials Provided (Cat. No. 025413)

2 x 1 ml	CONTROL LEVEL 1 Buffered bovine serum albumin containing approximately 50 pg/mL Intact PTH (Lyophilized). See vial label for the assigned concentration range.
2 x 1 ml	CONTROL LEVEL 2 Buffered bovine serum albumin containing approximately 800 pg/mL Intact PTH (Lyophilized). See vial label for the assigned concentration range.

Warnings and Precautions

- The AIA-PACK Intact PTH CONTROL SET is for IN VITRO DIAGNOSTIC USE ONLY.
- The control material has been tested by FDA approved methods and found negative for the presence of HBsAg and antibody to HIV-1 and HCV. Because not 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 controls accurately to the volume of 1 mL with CAP Class I or NCCLS (CLSI) Type 1 Reagent Grade water. Allow the lyophilized material to fully dissolve. Bring control to 18-25 °C for use. Always store the Control 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 Intact PTH CONTROL SET is stable until the expiration date on the label. Control materials should be used within 7 days of opening or reconstituting provided that the vials are kept sealed and refrigerated at 2-8 °C.

Procedure

NOTE: Refer to the AIA System Operator's Manual for additional procedural instructions regarding quality control.

1. Load the appropriate amount of ST AIA-PACK Intact PTH test cups on the instrument.
2. Add the appropriate amounts of each control to sample cups (refer to the instrument worksheet for the sample volume).
3. Print a work list and place the sample cups in the position indicated.

Assignment of Values

The AIA-PACK Intact PTH CONTROL SET contains an assigned concentration range of Intact PTH. The assigned range is determined on a lot-to-lot basis and is designed to provide target control levels of approximately 50 and 800 pg/mL of Intact PTH. Since the assay values are dependent upon assay procedures as well as several other factors, each laboratory should establish its own range for the assay procedure being monitored.

Limitations

The AIA-PACK Intact PTH CONTROL 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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