AIA-360 Assay Specifications
ST ACTH Test Code 106

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	300 pg/mL (example)
6	Cal 5	800 pg/mL (example)
7	Cal 6	2200 pg/mL (example)
8	Cal Lot L	
9	Cal Lot R	
10	Unit	pg/mL
11	Smpl. Vol	50
12	Dil. Vol	50
13	Assay L	2.00
14	Assay H	2000.00
15	Ref. L	
16	Ref. H	
17	Decimal	2
	Visible only in Test Mode	
18	Test Name	#ACTH
19	Calib. No	6
20	Calib. Mul	3
21	Calib. Equ	3
22	Calib. CV	90
23	Assay Prtl	1
24	Factor1 A	1.00
25	Factor1 B	0.00
26	Factor2 A	1.00
27	Factor2 B	0.00
28	V. Conc	0.00
29	G Origin	0.00

AIA-900 Assay Specifications
ST ACTH Test Code 106

No.	Item	Data
1	CODE	106
2	ACT	1
3	ANALYTE	#ACTH
4	LOT	***Enter current lot number
5	CALIB. 1	0 pg/mL
6	CALIB. 2	15 pg/mL (example)
7	CALIB. 3	50 pg/mL (example)
8	CALIB. 4	300 pg/mL (example)
9	CALIB. 5	800 pg/mL (example)
10	CALIB. 6	2200 pg/mL (example)
11	CALLOT L	000
12	CALLOT R	000
13	UNIT	pg/mL
14	DECIMAL	2
15	ASSAY L	2.0
16	ASSAY H	2000.0
17	REF L	
18	REF H	
19	RESCHED. L	2.0
20	RESCHED. H	2000.0
21	FACTOR A	1.00
22	FACTOR B	0.00
23	SMPL. VOL.	50
24	DIL. VOL.	50
25	2 REAG	0
26	CAL. CODE	106
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	1
34	DIL. CNTL.	1
35	DIL. DO	1
36	DIL. AH	5
37	DIL. CALC	1
38	DIL. CODE	106
39	DIL. NAME	ACTH
40	DIL. PRTY	3
41	PRE. SPVOL	0
42	PRE. 1VOL	0
43	PRE. 2VOL	0
44	PRE.CODE	000
45	PRE.NAME	
46	PROTOCOL	1
47	SYS. F A	1.00
48	SYS. F B	0.00
49	V. CONC.	0
50	G. ORIGIN	0

AIA-2000 Assay Specifications
ST ACTH Test Code 106

No.	Item	Data
1	Unit	pg/mL
2	Decimal places	2
3	Reference low	
4	Reference high	
5	Reschedule low	2.0
6	Reschedule high	2000.0
7	Assay range low	2.00
8	Assay range high	2000.00
9	Specimen diluent code	106
10	Specimen diluent name	ACTH
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	1.00
17	Factor B	0.00
18	Calibration code	3
19	Calibrator replicates	3
20	Calibrator lot	***Enter current lot number
21	Calibrator Concentration	
22	Cal - 1	0 pg/mL
23	Cal - 2	15 pg/mL (example)
24	Cal - 3	50 pg/mL (example)
25	Cal - 4	300 pg/mL (example)
26	Cal - 5	800 pg/mL (example)
27	Cal - 6	2200 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	50
39	Diluent volume	50
40	Conjugate volume	0
41	Diluted conjugate volume	0
42	Pretreatment	No
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	106
50	Virtual Concentration	0.0
51	Graph Origin	0.0
52	Calculation with Dilution Factor	Yes

Adrenocorticotropic Hormone
ST AIA-PACK ACTH

Name and Intended Use

ST AIA-PACK ACTH is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of Adrenocorticotropic hormone (ACTH) in human EDTA plasma. Measurements of ACTH are used in the differential diagnosis and treatment of certain disorders of the adrenal glands such as Cushing's syndrome, adrenocortical insufficiency and the ectopic ACTH syndrome

Summary and Explanation of Test

Adrenocorticotropic hormone (ACTH) is a non-glycosilated 39-amino acid polypeptide hormone [MW 4,541 Daltons]. ACTH is processed from pro-opiomelanocortin (POMC) in the anterior pituitary gland and is secreted to blood.[1,2]
The main biological function of ACTH is to increase the synthesis and release of all adrenal steroid hormones.

ACTH secretion from the anterior pituitary gland is mainly controlled by the corticotropin releasing hormone (CRH) and the adrenal steroid hormones (negative feedback). CRH is secreted from the hypothalamus and promotes ACTH synthesis and release. Since the secretion of CRH is based on circadian rhythm, ACTH levels fluctuate; high levels are secreted in the morning and low levels are secreted in the evening. Based on such circadian rhythm, it is recommended that blood be collected in the early morning for ACTH analysis. Furthermore, ACTH levels increase under various types of stress. Stress reinforces the synthesis of CRH and antidiuretic hormone (ADH) or vasopressin. The increased ADH enhances the stimulation of CRH to release ACTH.

Secreted ACTH acts on the adrenal cortex to synthesize and release adrenal steroid hormones such as cortisol. Cortisol at high concentrations in blood acts on the hypothalamus and pituitary gland, and inhibits the synthesis and release of CRH, and consequently ACTH (negative feedback).

Principle of the Assay

The ST AIA-PACK ACTH is a two-site immunoenzymometric assay which is performed entirely in the ST AIA-PACK ACTH test cups. ACTH present in the test sample is bound with anti-ACTH goat polyclonal antibody immobilized on magnetic beads and enzyme-labeled anti-ACTH goat polyclonal antibody. The magnetic beads are washed to remove unbound enzyme-labeled anti-ACTH goat polyclonal antibody and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The enzyme alkaline phosphatase causes oxidation of 4MUP to 4MU. 4MU is excited at 365 nm and comes to ground state at 448 nm releasing fluorescent energy. The amount of fluorescent energy is measured by the detector.

The amount of enzyme-labeled anti-ACTH goat polyclonal antibody that binds to the beads is directly proportional to the ACTH concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve.

Material Provided (ST AIA-PACK ACTH, Cat No. 0025221)

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

The following materials are required to perform Adrenocorticotropic hormone analysis using the ST AIA-PACK ACTH (Cat. No. 0025221) on the TOSOH AIA System Analyzer. The following are available separately from TOSOH.

Materials Required But Not Provided

The following materials are not provided but are required to perform ACTH analysis using the ST AIA-PACK ACTH (Cat. No. 0025221) on the Tosoh AIA System Analyzer. They are available separately from Tosoh.

Materials		Cat. No.
AIA-SYSTEMS:		
AIA-2000 ST		0022100
AIA-2000(LA)		0022101
AIA-360		0019945
AIA-900		0022930
AIA-PACK		
AIA-PACK SUBSTRATE SET II		0020968
AIA-PACK SUBSTRATE REAGENT II / AIA-PACK SUBSTRATE RECONSTITUENT II		
ST AIA-PACK ACTH CALIBRATOR SET		0025321
ST AIA-PACK ACTH CALIBRATOR(1)	0 pg/mL	
ST AIA-PACK ACTH CALIBRATOR(2)	15 pg/mL (approx.)	
ST AIA-PACK ACTH CALIBRATOR(3)	50 pg/mL (approx.)	
ST AIA-PACK ACTH CALIBRATOR(4)	300 pg/mL (approx.)	
ST AIA-PACK ACTH CALIBRATOR(5)	800 pg/mL (approx.)	
ST AIA-PACK ACTH CALIBRATOR(6)	2200 pg/mL (approx.)	
ST AIA-PACK ACTH SAMPLE DILUTING SOLUTION		0025521
AIA-PACK ACTH CONTROL SET		0025421
CONTROL LEVEL 1 50 pg/mL (approx.)		
CONTROL LEVEL 2 300 pg/mL (approx.)		
AIA-PACK WASH CONCENTRATE		0020955
AIA-PACK DILUENT CONCENTRATE		0020956
SAMPLE CUPS		0018581
AIA-PACK DETECTOR STANDARDIZATION TEST CUPS		0020970
ADDITIONAL REQUIREMENTS :		
AIA-PACK SAMPLE TREATMENT CUPS		0020971
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 ACTH is intended for in vitro diagnostic use only.
- ℞ only. US Federal law restricts this device to sale by or on the order of a licensed healthcare practitioner.
- Inspect the packaging and the exterior of the aluminum pouch or the vials for any sign of damage before use. If any damages are visible, contact your local Tosoh sales representative.
- Test cups from different lots or different assays should not be mixed within a tray.
- The ST AIA-PACK ACTH 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 EDTA plasma 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 EDTA plasma, please use standard laboratory safety procedures in handling all specimens and controls.
- For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state, and federal regulations.
- Do not use beyond the expiration date.
- The ST AIA-PACK ACTH has been designed so that the high dose "hook effect" is not a problem for the vast majority of samples. Samples with ACTH concentrations between 2,000 and 1,000,000 pg/mL will read > 2,000 pg/mL. The "hook effect" phenomenon may occur only at ACTH concentrations > 1,000,000 pg/mL.
- Serum, dust, metal, or microorganism contamination may cause degradation of reconstituted substrate solution. Store in a clean environment, away from direct sunlight and ultraviolet light.

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 ACTH	0025221
ST AIA-PACK ACTH CALIBRATOR SET	0025321
ST AIA-PACK ACTH SAMPLE DILUTING SOLUTION	0025521
AIA-PACK SUBSTRATE SET II	0020968
AIA-PACK WASH CONCENTRATE	0020955
AIA-PACK DILUENT CONCENTRATE	0020956
Room Temperature (18 - 25 °C):	
AIA-PACK DETECTOR STANDARDIZATION TEST CUP	0020970
AIA-PACK SAMPLE TREATMENT CUP	0020971

ST AIA-PACK ACTH test cups may be stored for up to 1 day at 18 - 25 °C. ST AIA-PACK ACTH CALIBRATOR SET must be kept tightly sealed and refrigerated at 2 - 8 °C. After opening, the calibrators are best recommended to be used once and for all within 1 day. After opening, ST AIA-PACK ACTH SAMPLE DILUTING SOLUTION should be used within 7 days as far as it is opened for up to 7 hours at 18 - 25 °C in a day and the bottles are kept tightly sealed and refrigerated at 2 - 8 °C immediately after use. The sample diluting solution can be used for up to 90 days provided that 1) it is used for manual dilution 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

- EDTA plasma is required for the assay. Serum, heparinized or citrated plasma SHOULD NOT BE USED.
- A venous blood sample is collected aseptically with EDTA. Centrifuge and separate plasma from the packed cells as soon as possible.
- EDTA plasma samples may be stored at 2 - 8 °C for up to 5 hours prior to analysis. If the analysis cannot be done within 5 hours, the sample should be stored frozen at -20 °C or below for up to 60 days.
- Repeated freeze-thaw cycles should be avoided because they may compromise results. Turbid EDTA plasma samples or samples containing particulate matter should be centrifuged prior to testing. Prior to assay, slowly bring frozen samples to 18 - 25 °C and mix gently. Samples should be measured within 2 hours when stored at 18 - 25 ℃.
- Hemolyzed sample should be avoided since proteases from red blood cells may cause degradation of ACTH.
- The sample required for analysis is 50 µL.

Procedure

1) Common Reagent Preparation

1a) Substrate Solution

Bring all reagents to 18 - 25 °C before preparing the working reagent. Add the entire contents of the AIA-PACK SUBSTRATE RECONSTITUENT II (100 mL) to the AIA-PACK SUBSTRATE REAGENT II (Lyophilized), mix thoroughly to dissolve the solid material.

1b) Wash Solution

Add the entire contents of the AIA-PACK WASH CONCENTRATE (100 mL) to approximately 2.0 L of CAP Class I water or the clinical laboratory reagent water, mix well, and adjust the final volume to 2.5 L.

1c) 1c) Diluent

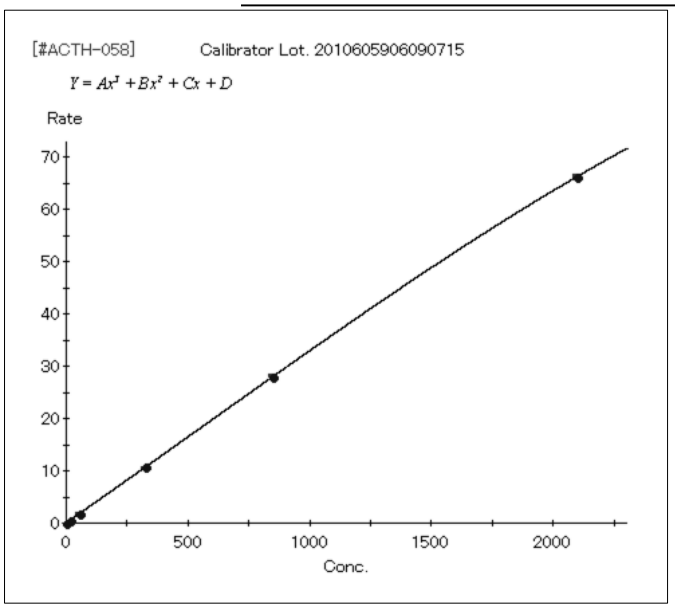
Add the entire contents of the AIA-PACK DILUENT CONCENTRATE (100 mL) to approximately 4.0 L of CAP Class I water or the clinical laboratory reagent water, mix well, and adjust the final volume to 5.0 L.

2) Calibration

2a) Calibration Curve

The calibrators for use with the ST AIA-PACK ACTH are prepared gravimetrically and compared to internal reference standards.
The calibration curve for the ST AIA-PACK ACTH is stable for up to 90 days. Calibration stability is monitored by quality control performance and is dependent on proper reagent handling and TOSOH 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 when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe or detector lamp adjustment or change).
For further information regarding instrument operation, consult the TOSOH AIA System Operator’s Manual.

A sample calibration curve from the AIA-2000 follows and shows the algorithm used for calculating results.



2b) Calibration Procedure

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

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

ii) The ST AIA-PACK ACTH CALIBRATOR (1) is provided ready for use.

iii) Calibrator levels (2)-(6) for ST AIA-PACK ACTH are lyophilized. Lyophilized calibrators should be reconstituted with 1.0 mL of CAP Class I water or the clinical laboratory reagent water.

iv) Tosoh recommends that all calibrators be run in triplicate.

2c) Calibration Acceptability Criteria

i) The mean rate for the CALIBRATOR (1) should be < 0.5 nmol/(L·s).

ii) Since there is a direct relationship between concentration and rate, the rate 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.

For further information regarding calibration, consult the Tosoh 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 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 Operator's Manual for the Tosoh AIA System Analyzer. In addition, refer to the Tosoh AIA System Operator's Manual for detailed instructions on defining and editing the files.

ii) Quality control should be run in accordance to local, state or federal regulations.

4) Specimen Processing

4a) Preparation

Following the specific instructions in the Operator's Manual for TOSOH AIA System Analyzer, place samples on the instrument appropriately. Barcoded primary tubes as well as sample cups can be run on the AIA Analyzer.

4b) Assay Procedure

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

ii) Load patient samples as instructed in the Operator's Manual and proceed with analysis. Note: The AIA Analyzer (except AIA-360) will require AIA-PACK SAMPLE TREATMENT CUPs if onboard dilutions are utilized.

Procedural Notes

1. Lyophilized Substrate must be completely dissolved.
2. Ligand assays performed by the Tosoh AIA System Analyzer require that the laboratory use water designated by the CAP Class I or by the clinical laboratory reagent water. 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 CLSI document GP40-A4-AMD, Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline - Fourth Edition.
3. If a specimen ACTH concentration is found to be greater than 2,000 pg/mL, the specimen should be diluted with the ST AIA-PACK ACTH SAMPLE DILUTING SOLUTION and reassayed according to the Assay Procedure. The recommended dilution for specimens containing greater than 2,000 pg/mL is 1:10 or 1:100. It is desirable to dilute the specimen so

that the diluted specimen reads between 2.0 and 2,000 pg/mL. The dilution factor should be entered into the software. For further information on the dilution of specimens, refer to the TOSOH AIA System Operator's Manual.

4. The Tosoh AIA System Analyzer can store two different calibration curves for each analyte at one time. Therefore, up to two different lots of ST AIA-PACK ACTH test cups can be used during the same run.
5. If the assay specifications for this test are not ready in the system software, the specifications must be entered under test code 106.

Calculation of Results

The Tosoh AIA System Analyzer performs all sample and reagent handling operations automatically. The Tosoh AIA System Analyzer reads the rate of fluorescence produced by the reaction and automatically converts the rate to ACTH concentration in pg/mL.

For samples requiring dilution AIA Analyzer (except AIA-360) will automatically perform dilutions and calculate results if the dilution factors are entered into the software. For detailed information regarding programming dilutions, consult the Tosoh AIA System 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 should be assayed daily.

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

1. After calibration, two levels of the control are run in order to accept the calibration curve.

2. 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).

3. 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 Analyzer.

If one or more control value(s) is out of the acceptable range, it will be necessary to investigate the cause.

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

Limitations of the Procedure

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 ACTH, the highest measurable concentration of ACTH in specimens without dilution is 2,000 pg/mL, and the lowest measurable concentration in specimens is 2.0 pg/mL (Assay Sensitivity).

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, 2,000 pg/mL.

ACTH concentration obtained from hemolyzed specimens may be lower than actual concentration since hemolyzed specimens contain proteases from red blood cells.

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

Samples from patients who had an injection of fluorescein, which is used in fluorescein fundus angiography, may cause falsely lowered results.

Certain medications may interfere with assay performance. Specimens from patients taking medicines and/or medical treatment may show erroneous results. All results should be interpreted with respect to the clinical picture of the patient.

Specimens containing heterophilic antibodies may give falsely elevated or decreased ACTH concentration.

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 corresponding 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 reference range study was developed with reference to the CLSI protocol entitled: How to Define and Determine Reference Intervals in the Clinical Laboratory (C28-A3).A total of 121 unaltered EDTA plasma specimens were assayed in singleton utilizing the ST AIA-PACK ACTH assay on the AIA-2000 analyzer. The specimens were collected from apparently healthy ambulatory individuals with no known history of adrenal or pituitary disease. Specimens from 110 males between the ages of 26 to 60 years and 11 females between the ages of 28 to 44 years were included in this study.

The reference range specimens had a normal distribution and the central 95% range was defined as the reference interval. The reference range for ST AIA-PACK ACTH is 7.4 to 64.3 pg/mL.

Number of Samples (n)	121
Reference Interval (Central 95th percentile)	7.4-64.3 pg/mL

Performance Characteristics

The following performance characteristics were determined using Tosoh AIA-2000 Automated Immunoassay Analyzer.

1) Recovery

1a) Dilution:

Two ACTH stock solutions (1,240,000 pg/mL and 375,000 pg/mL) were prepared by dissolving ACTH (Bachem AG, Cat. No. H-1160 # 1003186) with ST AIA-PACK ACTH SAMPLE DILUTING SOLUTION.
Unaltered EDTA plasma specimens (Plasma Specimens #1 -5) of known ACTH concentration were spiked with one of the ACTH stock solutions.
The following % of high ACTH specimens were diluted with ST AIA-PACK ACTH SAMPLE DILUTING SOLUTION: 75%, 50%, 25% and 10% and 5%.

Plasma Specimen ID	[ACTH] pg/mL	Dilution [% High ACTH Specimen]	Mean [ACTH] pg/mL	Expected [ACTH] pg/mL	% Recovery
Plasma-1: #TRET2K092	19,847	10	2,053	1,985	103
		5	996	992	100
Plasma-2: #TKET00812	21,083	10	2,023	2,108	96
		5	1,073	1,054	102
Plasma-3: #TKET00102	2,286	75	1679.6	1621.5	104
		50	1163.1	1081.0	108
		25	563.7	540.5	104
		10	219.7	216.2	102
Plasma 4: #TKET00346	2,114	75	1632.8	1637.3	100
		50	1087.3	1091.6	100
		25	529.1	545.8	97
		10	200.9	218.3	92
Plasma-5: #TKET11356	2,431	75	1689.7	1674.8	101
		50	1158.4	1116.5	104
		25	563.1	558.3	101
		10	229.6	223.3	103

1b) Linearity:

The linearity for ST AIA-PACK ACTH was determined, based on guidance from CLSI Protocol EP06-A. Two linearity studies were conducted; each study used two EDTA plasma specimens to determine the linearity. The assay has been demonstrated to be linear from 2.0 to 2000 pg/mL, within +/- 10% difference in this interval.

EDTA Plasma Study 1 (1.65 to 2560.97 pg/mL):

Dilution No.	Dilution Ratio Low : High	Expected Value (pg/mL)	Measured Value (pg/mL)		
			Mean	SD	CV (%)
1	Low Undiluted	1.65	1.65	0.05	3.2
2	9 : 1	257.59	251.05	5.57	2.2
3	8 : 2	513.52	480.09	12.28	2.6
4	7 : 3	769.45	757.99	10.50	1.4
5	6 : 4	1025.38	1014.03	26.43	2.6
6	5 : 5	1281.31	1270.33	12.51	1.0
7	4 : 6	1537.24	1555.37	28.76	1.8
8	3 : 7	1793.17	1769.56	46.99	2.7
9	2 : 8	2049.11	2062.87	30.31	1.5
10	1 : 9	2305.04	2279.89	47.52	2.1
11	High Undiluted	2560.97	2560.97	44.02	1.7

EDTA Plasma Study 2 (1.58 to 231.39 pg/mL):

Dilution No.	Dilution Ratio Low : High	Expected Value (pg/mL)	Measured Value (pg/mL)		
			Mean	SD	CV (%)
1	Low Undiluted	1.58	1.6	0.08	4.8
2	9 : 1	24.56	23.5	0.32	1.4
3	8 : 2	47.54	46.0	0.65	1.4
4	7 : 3	70.52	68.2	0.72	1.1
5	6 : 4	93.50	91.3	2.03	2.2
6	5 : 5	116.49	116.9	2.99	2.6
7	4 : 6	139.47	137.8	2.29	1.7
8	3 : 7	162.45	161.6	1.08	0.7
9	2 : 8	185.43	180.9	1.74	1.0
10	1 : 9	208.41	204.0	3.14	1.5
11	High Undiluted	231.39	231.4	2.91	1.3

EDTA Plasma Study 3 (1.9 to 2415.3 pg/mL):

Dilution No.	Dilution Ratio Low : High	Expected Value (pg/mL)	Measured Value (pg/mL)		
			Mean	SD	CV (%)
1	Low Undiluted	1.89	1.9	0.12	6.2
1.18	9.85:0.15	38.09	35.9	0.96	2.7
1.3	9.70:0.30	74.3	74.8	1.43	1.9
1.6	9.40:0.60	146.7	152	3.72	2.5
2	9:01	243.24	242.2	4.51	1.9
3	8:02	484.58	481.6	4.72	1
4	7:03	725.93	738.3	11	1.5
5	6:04	967.27	983.8	26.79	2.7
6	5:05	1208.62	1234.5	26.43	2.1
7	4:06	1449.96	1440.2	9.95	0.7
8	3:07	1691.3	1696.9	27.32	1.6
9	2:08	1932.65	1946.7	49.48	2.6
10	1:09	2173.99	2145.2	42.48	2
11	High Undiluted	2415.34	2415.3	75.21	3.1

2) Precision

The precision study was developed with reference to the CLSI protocol entitled: Evaluation of Precision Performance of Quantitative Measurement Methods (EP05-A2).

The precision study for the ST AIA-PACK ACTH assay was evaluated utilizing three AIA-2000 analyzers and 3 different lots of reagents. Precision was assessed by assaying three levels of unaltered EDTA plasma specimens. Estimates of total and within-run precision were obtained from measurements of 2 replicates in a single run, 2 times a day for 20 non-consecutive days. This equaled to a total of 40 runs and 80 determinants.

2a) Within run precision:

Within run precision CV% was estimated to be 1.5 to 3.1%.

Specimen	Reagent Set # 1			Reagent Set # 2			Reagent Set # 3		
	Mean (pg/mL)	Pooled SD	CV %	Mean (pg/mL)	Pooled SD	CV %	Mean (pg/mL)	Pooled SD	CV %
EDTA Plasma-A	37.8	1.2	3.1	44.3	1.2	2.8	40.9	0.89	2.2
EDTA Plasma-B	223.7	4.8	2.1	244.6	5.1	2.1	230.0	5.3	2.3
EDTA Plasma-C	709.2	10.9	1.5	740.4	15.6	2.1	719.1	15.5	2.2

2b) Total precision

Total precision CV% was estimated to be 2.2 to 4.2%.

Specimen	Reagent Set # 1			Reagent Set # 2			Reagent Set # 3		
	Mean (pg/mL)	Pooled SD	CV %	Mean (pg/mL)	Pooled SD	CV %	Mean (pg/mL)	Pooled SD	CV %
EDTA Plasma-A	37.8	1.2	3.3	44.3	1.9	4.2	40.9	1.03	2.5
EDTA Plasma-B	223.7	5.7	2.5	244.6	9.3	3.8	230.0	6.4	2.8
EDTA Plasma-C	709.2	15.6	2.2	740.4	25.1	3.4	719.1	15.9	2.2

Correlation

The methods comparison study was developed with reference to the CLSI protocol entitled: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (EP09-A2).

a. Alternate Method Comparison

A total of 160 EDTA plasma specimens (154 unaltered and 6 altered specimens) were assayed in singleton utilizing the ST AIA-PACK ACTH assay on the AIA-2000 analyzer and the alternate method (x). A combination of fresh and frozen specimens was utilized for this study. Six of the 160 specimens were mixed using two or more specimens and altered.

Regression Analysis		
	Deming	Regular
Slope:	1.10 (1.075 to 1.116)	1.09 (1.067 to 1.107)
Intercept:	-0.84 (-8.921 to 7.234)	0.76 (-7.305 to 8.817)
95% Confidence Intervals are shown in parentheses		
Corr Coef (R):	0.993	
Bias: (all specimens)	17.8 pg/mL	
Points (Plotted/Total):	160/160	
Result Ranges:	3.8 to 1986 pg/mL	

Limit of Detection (LoD) and Limit of Quantitation (LoQ)

The Limit of Detection (LoD) for AIA-PACK ACTH is 0.7pg/mL, determined consistent with the guidelines in the CLSI protocol EP 17-A based on 120 determinations, with 60 blank and 60 low level samples. LoB is 0.5pg/mL. LoQ is 1.2pg/mL, but the low end sensitivity claim for the assay is conservatively set at 2.0 pg/mL.

The calculation of LoQ was as follows:

Low level samples were prepared by dilution of a high sample. The sample range was loB to 4 times LoB. The assigned value for these samples was based on the dilution factor. Samples were assayed 2 times a day for 5 days. The mean and SD were calculated for these 10 measurements. The bias was calculated as the difference between the assigned value and measured value. The bias was equal to -0.045 and CV was 3.4%.

A precision profile was plotted for the low level samples and the functional sensitivity at 20% CV was 0.683 pg/mL and at 10% CV was 1.377 pg/mL.

Specificity:

The recovery of ACTH should be within 90 - 110%.

ACTH Fragment	ACTH fragment conc. [pg/mL]	Measured ACTH conc. [pg/mL]			Difference (measured – original)	ACTH Recovery [%]	% Cross-reactivity
		Value -1	Value-2	Average			
(Control)	0	55.1	53.6	54.3	---	---	---
ACTH 1-10	500	54.4	54.2	54.3	0.0	100%	-0.01%
	5,000	55.0	54.4	54.7	0.4	101%	0.01%
	100,000	55.3	56.1	55.7	1.3	102%	0.00%
ACTH 11-24	500	53.5	53.4	53.4	-0.9	98%	-0.18%
	5,000	54.1	53.0	53.6	-0.8	99%	-0.02%
	100,000	53.8	53.8	53.8	-0.5	99%	0.00%
Beta-MSH	500	53.7	54.0	53.8	-0.5	99%	-0.10%
	5,000	54.1	54.9	54.5	0.2	100%	0.00%
	100,000	51.1	52.1	51.6	-2.8	95%	0.00%
Beta-Endorphin	500	53.1	53.0	53.0	-1.3	98%	-0.26%
	5,000	54.9	53.5	54.2	-0.1	100%	0.00%
	100,000	53.1	52.5	52.8	-1.5	97%	0.00%

ACTH 1-10, 11-24, beta-MSH and beta-Endorphin do not interfere the assay up to 100,000 pg/mL.

ACTH recovery was less than 90% in the presence of ACTH 1-17, 1-24, 18-39, alpha-MSH (>5,000pg/mL) or ACTH 22-39 (>100,000 pg/mL). These fragments may negatively affect the ACTH assay. It is likely that these fragments bind to either the antibodies on beads or the enzyme-labeled antibodies. Therefore, if these fragments were contained excessively in the test sample, lower values may be reported.

Interference

The criteria for no interference are +/- 10% (90-110%) recovery of ACTH of the known specimen mean concentration.

- Hemoglobin (up to 440 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 triglyceride concentration (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 concentration (up to 50 mg/mL), does not interfere with the assay.
- EDTA (up to 7 mg/mL) does not interfere with the assay.
- Rheumatoid factor (up to 500 IU/mL) does not interfere with the assay.
- Heparin (up to 50 U/mL) does not interfere with the assay.
- Acetaminophen (up to 20 mg/L) does not interfere with the assay.
- Acetylsalicylic acid (up to 300 mg/L) does not interfere with the assay.
- Ampicillin (up to 200 mg/L) does not interfere with the assay.
- Ibuprofen (up to 50 mg/L) does not interfere with the assay.
- Theophylline (up to 10 mg/L) does not interfere with the assay.

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2. Fenger M., et al., Amidated peptides derived from pro- opiomelanocortin in normal human pituitary. Biochem. J., 250, 781-788 (1988)

3. Castro M., et al., Out-Patient Screening for cushing's syndrome: The sensitivity of the combination of circadian rhythm and overnight dexamethasone suppression salivary cortisol tests. J Clin Endocrinol Metab, 84, 878-882 (1999)

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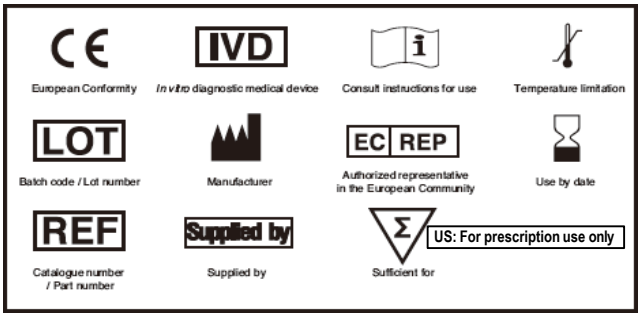
CE
European Conformity
IVD
In vitro diagnostic medical device
Consult instructions for use
Temperature limitation
LOT
Batch code / Lot number
Manufacturer
EC REP
Authorized representative in the European Community
Use by date
REF
Catalogue number / Part number
Supplied by
Supplied by
Σ
Sufficient for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

ST AIA-PACK
ACTH CALIBRATOR SET

Intended Use

The ST AIA-PACK ACTH CALIBRATOR SET is intended for IN VITRO DIAGNOSTIC USE ONLY for the calibration of the ST AIA-PACK ACTH assay.

Summary and Explanation

The ST AIA-PACK ACTH CALIBRATOR SET contains buffered bovine serum albumin with assigned levels of ACTH. ACTH from human origin is used in the preparation of these calibrators. Calibration should be performed according to the schedule indicated in the Tosoh AIA System Operator's Manual.

Material Provided (Cat. No. 0025321)

2 x 1 mL	ST AIA-PACK ACTH CALIBRATOR (1)	0.0	pg/mL

Buffered bovine serum albumin containing no detectable concentration of ACTH with 0.1% sodium azide as a preservative (ready for use).

2 x 1 mL	ST AIA-PACK ACTH CALIBRATOR (2)	15	pg/mL (approx.)
	ST AIA-PACK ACTH CALIBRATOR (3)	50	pg/mL (approx.)
	ST AIA-PACK ACTH CALIBRATOR (4)	300	pg/mL (approx.)
	ST AIA-PACK ACTH CALIBRATOR (5)	800	pg/mL (approx.)
	ST AIA-PACK ACTH CALIBRATOR (6)	2200	pg/mL (approx.)

Buffered bovine serum albumin containing the assigned concentration of ACTH (described on each vial) with 0.1% sodium azide as a preservative (lyophilized).

Warnings and Precautions

1. The ST AIA-PACK ACTH CALIBRATOR SET is for in vitro diagnostic use.
2. This material 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.
3. The material derived from human origin used in the preparations of these calibrators 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 origin will not transmit infectious agents, it is recommended that this product be handled with the same precautions as used for patient samples.
4. Do not use beyond the expiration date.

Preparation of Reagents

- Calibrator levels (2)-(6) for ST AIA-PACK ACTH CALIBRATOR SET are lyophilized. Lyophilized calibrators should be reconstituted with 1.0 mL of CAP Class I water or the clinical laboratory reagent water.
- Bring the calibrators to 18 - 25 °C for use.
- Using a volumetric pipette or a calibrated adjustable pipette, reconstitute the lyophilized calibrators accurately. Allow the lyophilized material to fully dissolve. Always store the Calibrator Set in an upright position at 2 - 8 °C when not in use.

Storage and Stability

When stored unopened and refrigerated at 2 - 8 °C, the ST AIA-PACK ACTH CALIBRATOR SET is stable until the expiration date on the label. Calibrator materials should be used within 1 day after opening, provided the vials are kept tightly sealed and refrigerated at 2 - 8 °C.

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.

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 ACTH CALIBRATOR SET contains assigned concentrations of ACTH. The assigned value is determined on a lot-by-lot basis and is designed to provide an assay calibration range of 2.0 to 2200 pg/mL of ACTH. The calibrators in this set are prepared gravimetrically and are compared to internal reference standards.

Results

1. The mean rate for the CALIBRATOR (1) should be < 0.5 nmol/(L·s).
2. Since there is a direct relationship between concentration and rate, the rate should increase as the concentration increases.
3. The replicate values should be within a 10 % range.

Limitations

The ST AIA-PACK ACTH CALIBRATOR SET is designed solely for use with ST AIA-PACK ACTH assay procedures. Although the approximate value of the highest calibrator is 2200 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.

References

1. AIA Analyte Application Manual (AAM). Tosoh Bioscience, Inc., Grove City, OH.
2. AIA-System Operator's Manual. Tosoh Bioscience, Inc., Grove City, OH.

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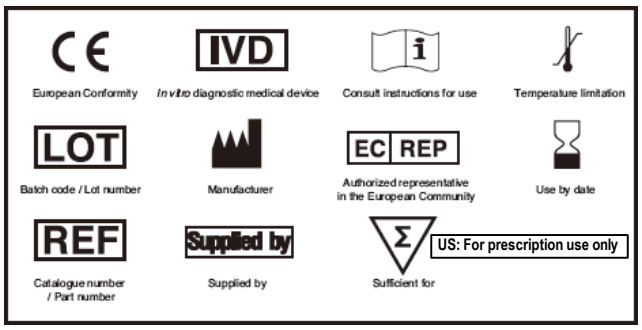


ST AIA-PACK
ACTH SAMPLE DILUTING SOLUTION

Intended Use

The ST AIA-PACK ACTH SAMPLE DILUTING SOLUTION is intended for IN VITRO DIAGNOSTIC USE ONLY to dilute patient samples.

Summary and Explanation

The ST AIA-PACK ACTH SAMPLE DILUTING SOLUTION contains a bovine protein matrix with no detectable concentration of ACTH. This sample diluting solution is to be used only with samples that are being tested for ACTH concentrations using the ST AIA-PACK ACTH assay.

Materials Provided (Cat. No. 0025521)

4 x 4mL	ST AIA-PACK ACTH SAMPLE DILUTING SOLUTION Buffered bovine serum albumin containing no detectable concentration of ACTH with sodium azide as a preservative.

Warnings and Precautions

1. The ST AIA-PACK ACTH SAMPLE DILUTING SOLUTION is intended for in vitro diagnostic use.
2. This material 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.
3. Although material derived from human origin is not used for the sample diluting solution, it is recommended that this product be handled with the same precautions as used for patient samples.
4. Do not use beyond the expiration date.

Preparation and Storage

- The ST AIA-PACK ACTH SAMPLE DILUTING SOLUTION is provided ready for use.
- Always store the sample diluting solution upright as refrigerated at 2-8°C when not in use.

Stability

When stored unopened and refrigerated at 2 - 8 °C, ST AIA-PACK ACTH SAMPLE DILUTING SOLUTION is stable until the expiration date on the label. The sample diluting solution should be used within 7 days as far as it is opened for up to 7 hours at 18 - 25 °C in a day and the bottles are kept tightly sealed and refrigerated at 2 - 8 °C immediately after use. They should be used after equilibrating to 18 - 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 bottles are kept tightly sealed and refrigerated immediately after use.

Procedure

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

1. If a specimen ACTH concentration is found to be greater than 2000 pg/mL, the specimen should be diluted with the ST AIA-PACK ACTH SAMPLE DILUTING SOLUTION and assayed according to the Procedure in the insert sheet of the ST AIA-PACK ACTH.
2. The AIA Analyzer (except AIA-360) will perform dilutions automatically if the dilution factors are entered into the software prior to assaying the diluted sample.
3. The recommended dilution for specimens containing greater than 2000 pg/mL is 5 fold dilution. It is desirable to dilute the specimen so that the diluted specimen reads between 2.0 and 2000 pg/mL.

Results

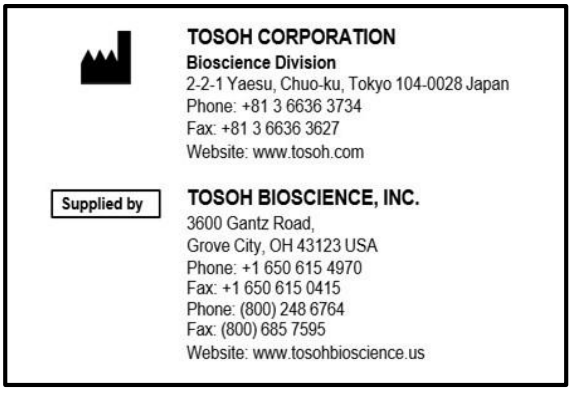
When an auto-dilution is performed, the AIA instrument will calculate the final result.

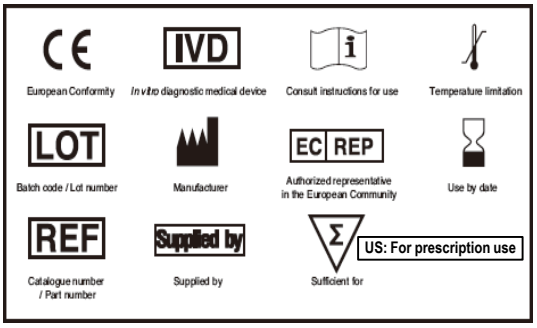
Limitations

The ST AIA-PACK ACTH SAMPLE DILUTING SOLUTION is designed solely for use with ST AIA-PACK ACTH assay procedures.

References

1. AIA Analyte Application Manual (AAM). Tosoh Bioscience, Inc., Grove City, OH.
2. AIA-System Operator's Manual. Tosoh Bioscience, Inc., Grove City, OH.





AIA-PACK
ACTH CONTROL SET

Intended Use

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

CL AIA-PACK values listed on the Control product labeling (Box and Vials) are for International Use Only and are not intended for use with products in the United States.

Summary and Explanation

The AIA-PACK ACTH CONTROL SET contains buffered bovine serum albumin with the assigned levels of ACTH.
After calibration, controls are run in order to confirm the calibration curve.
Controls are repeated if 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.

Materials Provided (Cat. No. 0025421)

2 x 1 mL	AIA-PACK ACTH CONTROL LEVEL 1	Buffered bovine serum albumin containing approximately 50 pg/mL ACTH (Lyophilized). See vial label for the assigned concentration range.
2 x 1 mL	AIA-PACK ACTH CONTROL LEVEL 2	Buffered bovine serum albumin containing approximately 300 pg/mL ACTH (Lyophilized). See vial label for the assigned concentration range.

Warnings and Precautions

- The AIA-PACK ACTH CONTROL SET is intended for IN VITRO DIAGNOSTIC USE ONLY.
- The control material 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.
- AIA-PACK ACTH CONTROL SET has been designed so that ST AIA-PACK ACTH gives precise results.

Preparation and Storage

- Using a volumetric pipette or a calibrated adjustable pipette, reconstitute the lyophilized controls accurately to the volume of 1 mL with CAP Class I water or the clinical laboratory reagent water. Allow the lyophilized material to fully dissolve.
- Bring controls to 18 - 25 °C for use.
- Always store the controls 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 ACTH 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 tightly sealed and refrigerated at 2 - 8 °C.

Procedure

Refer to the Tosoh AIA System Operator's Manual for additional procedural instructions regarding quality control. Refer to federal, state and local guidelines regarding quality control procedures.

1. Load the appropriate amount of ST AIA-PACK ACTH test cups on the instrument.
2. Add the appropriate amount of each control to sample cups. (Refer to the instrument worksheet for the sample volume.)
3. Print a worklist and place the sample cups in the position indicated.

Assignment of Values

The AIA-PACK ACTH CONTROL SET contains assigned concentration range of ACTH. The assigned range is determined on a lot-by-lot basis and is designed to provide target control levels of approximately 50 and 300 pg/mL of ACTH. The values are assigned by testing two lots of controls in five replicates. The grand mean and %CV are determined, and the range is established as +/- 20% of the grand mean.

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 ACTH CONTROL SET is designed solely for use with ST AIA-PACK ACTH assay procedures.

References

1. AIA Analyte Application Manual (AAM). Tosoh Bioscience, Inc., Grove City, OH.
2. AIA-System Operator's Manual Tosoh Bioscience, Inc., Grove City, OH.