AIA-360 Assay Specifications
ST PROG Test Code 037

No.	Item	Data
1	Calib. Req	Show
2	Cal 1	0 ng/mL
3	Cal 2	0.5 ng/mL (example.)
4	Cal 3	1.5 ng/mL (example.)
5	Cal 4	5.0 ng/mL (example.)
6	Cal 5	15 ng/mL (example.)
7	Cal 6	45 ng/mL (example.)
8	Cal Lot L	
9	Cal Lot R	
10	Unit	ng/mL
11	Smpl. Vol	75
12	Dil. Vol	75
13	Assay L	0.10
14	Assay H	40
15	Ref. L	
16	Ref. H	
17	Decimal	2
	Visible only in Test Mode	
18	Test Name	#PROG
19	Calib. No	6
20	Calib. Mul	3
21	Calib. Equ	6
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 PROG Test Code 037

Screen	Item	Data
Screen 1		
	Lot	***Enter current cal. lot no.
	Cal 1	0 ng/mL
	Cal 2	0.5 ng/mL (example.)
	Cal 3	1.5 ng/mL (example.)
	Cal 4	5.0 ng/mL (example.)
	Cal 5	15 ng/mL (example.)
	Cal 6	45 ng/mL (example.)
Screen 2		
	Name	#PROG
	Unit	ng/mL
	Sample	75
	Dil	75
	2Reag	0
	Code	0
	Assay Range Low	0.10
	Assay Range High	40
	Reference Range Low	
	Reference Range High	
	DP (No. of decimal points)	2
Screen 3		
	Code	22
	No. (Calibrators)	6
	Mul. (Replicates)	3
	Equ	6
	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)	22
	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 PROG Test Code 037

No.	Item	Data
1	Code	37
2	ACT	0
3	Analyte	#PROG
4	Lot	***Enter current cal lot no.
5	CAL 1	0 ng/mL
6	CAL 2	0.5 ng/mL (example.)
7	CAL 3	1.5 ng/mL (example.)
8	CAL 4	5.0 ng/mL (example.)
9	CAL 5	15 ng/mL (example.)
10	CAL 6	45 ng/mL (example.)
11	Cal lot L	
12	Cal lot R	
13	Unit	ng/mL
14	Decimal	2
15	Assay Low	0.1
16	Assay High	40
17	Reference Low	
18	Reference High	
19	Reschedule Low	0.1
20	Reschedule High	40
21	Factor A	1
22	Factor B	0
23	Sample Volume	75
24	Diluent Volume	75
25	2 Step reagent dispensing volume	0
26	Calibration code	22
27	CAL. No.	6
28	CAL. MUL.	3
29	CAL. EQU.	6
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.	5
37	DIL. CALC.	1
38	DIL. CODE	22
39	DIL. NAME	PROG
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 PROG Test Code 037

No.	Item	Data
1	Unit	ng/mL
2	Decimal places	2
3	Reference low	
4	Reference high	
5	Reschedule low	0.10
6	Reschedule high	40
7	Assay range low	0.10
8	Assay range high	40
9	Specimen diluent code	22
10	Specimen diluent name	PROG
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	5
16	Factor A	1.00000e+000
17	Factor B	0.00000e+000
18	Calibration code	6
19	Calibrator replicates	3
20	Calibrator lot	***Enter current cal. lot no.
21	Calibrator Concentration	
22	Cal - 01	0 ng/mL
23	Cal - 02	0.5 ng/mL (example.)
24	Cal - 03	1.5 ng/mL (example.)
25	Cal - 04	5.0 ng/mL (example.)
26	Cal - 05	15 ng/mL (example.)
27	Cal - 06	45 ng/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	75
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	022
50	Virtual Concentration	0.0
51	Graph Origin	0.0
52	Calculation with Dilution Factor	Yes

AIA-2000 Assay Specifications
ST PROG Test Code 037

No.	Item	Data
1	Unit	ng/mL
2	Decimal places	2
3	Reference low	
4	Reference high	
5	Reschedule low	0.10
6	Reschedule high	40
7	Assay range low	0.10
8	Assay range high	40
9	Specimen diluent code	22
10	Specimen diluent name	PROG
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	5
16	Factor A	1.00000e+000
17	Factor B	0.00000e+000
18	Calibration code	6
19	Calibrator replicates	3
20	Calibrator lot	***Enter current cal. lot no.
21	Calibrator Concentration	
22	Cal - 01	0 ng/mL
23	Cal - 02	0.5 ng/mL (example.)
24	Cal - 03	1.5 ng/mL (example.)
25	Cal - 04	5.0 ng/mL (example.)
26	Cal - 05	15 ng/mL (example.)
27	Cal - 06	45 ng/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	75
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	022
50	Virtual Concentration	0.0
51	Graph Origin	0.0
52	Calculation with Dilution Factor	Yes

Progesterone
ST AIA-PACK PROG

Name and Intended Use

ST AIA-PACK PROG is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of Progesterone in human serum or heparinized plasma on specific Tosoh AIA System analyzers.

Summary and Explanation of Test

Progesterone (pregn-4-ene-3, 20-dione) is a steroid hormone produced mainly by the ovary and adrenal cortex in females and, in lesser amounts, by the adrenal cortex in males.[1,2] Progesterone is secreted in small amounts by the ovaries during the follicular phase of the menstrual cycle and increases sharply following ovulation and corpus luteum development.[3] Unless pregnancy occurs, a steep decline to follicular levels occurs prior to the next menstrual cycle.[4] This pattern provides the rationale for the use of serum progesterone measurements as a reliable method for ovulation detection.[5]

Daily progesterone levels are considered the most accurate means for documenting luteal phase defect.[6] Measurements of serum progesterone can also be used to detect and evaluate patients at risk for abortion during the early weeks of gestation.[7,8]

Principle of the Assay

The ST AIA-PACK PROG is a competitive enzyme immunoassay which is performed entirely within the AIA-PACK. Progesterone present in the test sample competes with enzyme-labeled progesterone for a limited number of binding sites on a progesterone-specific antibody immobilized on magnetic beads. The beads are washed to remove the unbound enzyme labeled progesterone and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme labeled progesterone that binds to the beads is inversely proportional to the progesterone concentration in the test sample. A standard curve using a range of known standard concentrations is constructed and unknown progesterone concentrations are calculated using this curve.

Material Provided (AIA-PACK PROG, Cat. No. 025281)

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

Plastic test cups containing lyophilized magnetic beads with anti-progesterone rabbit polyclonal antibody and progesterone 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 progesterone analysis using the ST AIA-PACK PROG (Cat. No. 025281) 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 PROG Calibrator Set	020381
Calibrator #1 0 ng/mL	
Calibrator #2 0.5 ng/mL (approx.)	
Calibrator #3 1.5 ng/mL (approx.)	
Calibrator #4 5.0 ng/mL (approx.)	
Calibrator #5 15 ng/mL (approx.)	
Calibrator #6 45 ng/mL (approx.)	
AIA-PACK PROG Sample Diluting Solution	020581
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 PROG is intended for in vitro diagnostic use only.
- Test cups from different lots should not be mixed within a tray.
- The ST AIA-PACK PROG 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 PROG	025281
AIA-PACK PROG Calibrator Set	020381
AIA-PACK PROG Sample Diluting Solution	020581
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 PROG 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. After opening, Sample Diluting Solution is stable for up to 90 days refrigerated at 2° - 8° C. 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, slowly bring frozen samples to room temperature (18° - 25° C) and mix gently. The sample required for analysis is 75µL.

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

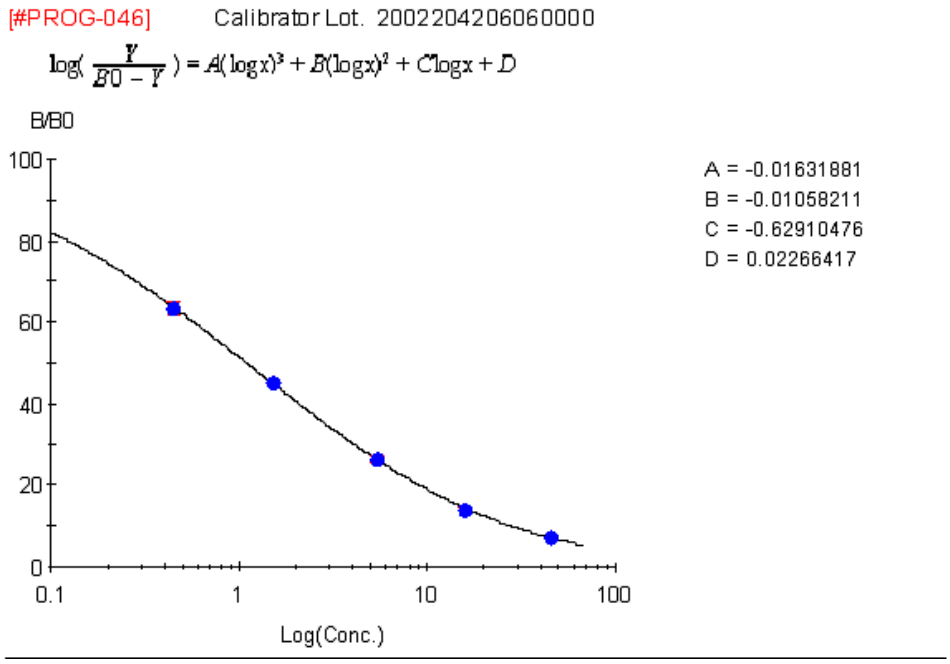
2a) Calibration Curve

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

The calibration curve for the ST AIA-PACK PROG 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 PROG are provided ready to use and need no reconstitution. 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 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 in the appropriate positions.

4b) Assay Procedure

i) Verify a sufficient quantity of ST AIA-PACK PROG 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. 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 progesterone concentration is found to be greater than the linearity limit of the assay, 40 ng/mL; the specimen should be diluted with the AIA-PACK PROG Sample Diluting Solution and re-assayed according to the Assay Procedure. The recommended dilution for samples containing greater than 40 ng/mL is no higher than 1:5. It is desirable to dilute the sample so that the diluted sample reads between 2.0 and 20.0 ng/mL. 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.
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 PROG 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 037.

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 progesterone concentration in ng/mL.

For samples requiring dilution, the AIA 900, AIA-600 II, AIA-1800 and AIA-2000 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, 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 strictest 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 PROG, the highest concentration of progesterone measurable without dilution is approximately 40 ng/mL, and the lowest measurable concentration is 0.1 ng/mL (assay sensitivity).

Although the approximate value of the highest calibrator is 45 ng/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, 40 ng/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.

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.[9]

Reference Ranges

The intervals given here were determined in serum samples from apparently healthy individuals.

Category	Samples	ng/mL	nmol/L
Male	23	<0.1 - 0.60 ng/mL	(< 0.318 - 1.91 nmol/L)
Ovulating Female			
Follicular phase	74	< 1.0 ng/mL	(< 3.18 nmol/L)
Luteal phase	61	2.50 - 34.0 ng/mL	(8.0 - 108.1 nmol/L)
Postmenopausal Female	23	<0.1 - 0.45 ng/mL	(<0.318 - 1.43 nmol/L)

Conversion Factors

Progesterone concentrations in this application are in units of ng/mL. Conversion to SI units of nmol/L may be made using the following equation:

nmol progesterone/L = ng progesterone/mL x 3.18

Performance Characteristics

1) Accuracy

1a) Recovery:

Three serum pools were spiked with three different levels of progesterone and assayed before and after spiking.

Sample	Initial Value (ng/mL)	PROG Added (ng/mL)	Expected Value (ng/mL)	Measured Value (ng/mL)	Percent Recovery (%)
Serum A1	1.56	2.97	4.53	4.44	98.0
	1.56	5.94	7.50	7.10	94.6
	1.56	11.84	13.40	13.02	97.1
Serum B1	0.18	2.97	3.15	2.88	91.2
	0.18	5.94	6.12	5.51	90.1
	0.18	11.84	12.02	10.97	91.2
Serum C1	1.23	2.97	4.20	4.49	106.9
	1.23	5.94	7.17	7.65	106.8
	1.23	11.84	13.07	13.64	104.4

1b) Dilution:

Three serum samples containing progesterone were serially diluted with AIA-PACK PROG Sample Diluting Solution and assayed.

Sample	Dilution Factor	Expected Value (ng/mL)	Measured Value (ng/mL)	Percent Recovery (%)
Serum A2	none		22.40	
	7.5/10	16.80	17.32	103.1
	5.0/10	11.20	10.55	94.2
	2.5/10	5.60	5.23	93.3
	1.0/10	2.24	1.95	87.1
Serum B2	none		25.90	
	7.5/10	19.43	18.98	97.7
	5.0/10	12.95	12.44	96.1
	2.5/10	6.48	6.24	96.3
	1.0/10	2.59	2.35	90.7
Serum C2	none		25.53	
	7.5/10	19.14	19.49	101.8
	5.0/10	12.76	12.36	96.9
	2.5/10	6.38	5.94	93.1
	1.0/10	2.59	2.24	87.8

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 (CV).

Sample	Mean (ng/mL)	Pooled SD (ng/mL)	Coefficient of Variation (%)
Control A	2.07	0.205	9.9
Control B	9.69	0.427	4.4
Control C	25.02	1.194	4.8

2b) Inter-assay precision

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

Sample	Number of Replicates	Mean (ng/mL)	Standard Deviation (ng/mL)	Coefficient of Variation (%)
Control A	20	2.09	0.240	11.5
Control B	20	9.86	0.625	6.3
Control C	20	25.40	1.39	5.5

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/mL)	Pooled SD (ng/mL)	Coefficient of Variation (%)
Control A	2.07	0.233	11.3
Control B	9.69	0.667	6.9
Control C	25.02	1.557	6.2

Specificity

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

Compound	Cross-reactivity (%)
Progesterone	100.0
17α-Hydroxyprogesterone	3.2
11-Deoxycorticosterone	7.6
Pregnelone	0.05
Corticosterone	4.2
5β-Pregnan-3,20-dione	13.3
Testosterone	0.06
Cortisol	0.01
5α-Pregnan-3,20-dione	11.7

Sensitivity

The minimal detectable concentration (MDC) of progesterone is estimated to be 0.1 ng/mL. The MDC is defined as that concentration of progesterone 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 430 mg/dL), free bilirubin (up to 8mg/dL) and conjugated bilirubin (up to 2 mg/dL) do not interfere with the assay.
- Lipemia, as indicated by added triglyceride (up to 70 mg/dL), does not interfere with the assay.
- Protein, as indicated by added albumin (up to 5 g/dL), does not interfere with the assay.
- Ascorbic acid (up to 20 mg/dL) does not interfere with the assay.

References

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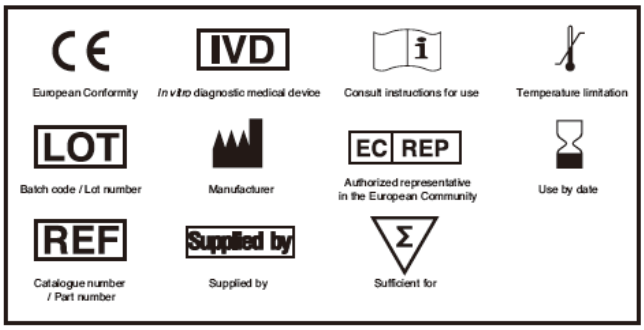

AIA-PACK
PROG Calibrator Set

Intended Use

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

Summary and Explanation

The AIA-PACK PROG Calibrator Set contains human serum with assigned levels of progesterone (PROG). Calibration should be performed according to the schedule indicated in the AIA System Operator's Manual. The calibrators in this set are prepared gravimetrically and are compared to internal reference standards.

Materials Provided (Cat. No. 020381)

2 x 1 mL	Calibrator	No. 1	0 ng/mL

Human serum containing no detectable concentration of progesterone (0 ng progesterone /mL), and 0.1% sodium azide as a preservative.

2 x 1 mL	Calibrator	No. 2	0.5 ng/mL (approx.)
		No. 3	1.5 ng/mL (approx.)
		No. 4	5.0 ng/mL (approx.)
		No. 5	15.0 ng/mL (approx.)
		No. 6	45.0 ng/mL (approx.)

Human serum containing the assigned concentration of progesterone (described on each vial), and 0.1% sodium azide as a preservative.

Warnings and Precautions

- The AIA-PACK PROG 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 approved 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

- The AIA-PACK PROG Calibrators are provided ready to use.
- 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 PROG 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 PROG Calibrator Set contains assigned concentrations of progesterone. The assigned value is determined on a lot-to-lot basis and is designed to provide an assay calibration range of approximately 0.0 to 45.0 ng/mL of progesterone. The calibrators in this set are prepared gravimetrically and are compared to internal reference standards.

Results

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

Limitations

The AIA-PACK PROG 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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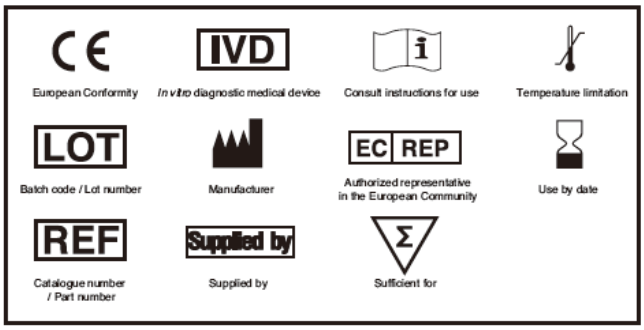

AIA-PACK
PROG Sample Diluting Solution

Intended Use

The AIA-PACK PROG Sample Diluting Solution is intended for IN VITRO DIAGNOSTIC USE ONLY to dilute patient samples that have concentrations of progesterone above the linear range of the assay.

Summary and Explanation

The AIA-PACK PROG Sample Diluting Solution contains human serum with no detectable concentration of progesterone. This Sample Diluting Solution is to be used only with samples that are being tested for progesterone concentrations using the ST AIA-PACK PROG assay.

Materials Provided (Cat. No. 020581)

4 x 4 mL	Sample Diluting Solution Human serum containing no detectable concentration of progesterone (0 ng progesterone/mL), and 0.1% sodium azide as a preservative.

Warnings and Precautions

- The AIA-PACK PROG 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.
- Human sera used in the preparation of this product has been tested by FDA approved 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

- The AIA-PACK PROG 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 AIA-PACK PROG Sample Diluting Solution is stable until the expiration date on the label. After opening, the Sample Diluting Solution is stable for up to 90 days when refrigerated at 2° - 8° C.

Procedure

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

1. If a specimen is found to contain greater than the linearity limit of approximately 45 ng/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.
2. The AIA 900, AIA-600 II, AIA-1800 and AIA-2000 will perform dilutions automatically if the dilution factors are entered into the software prior to assaying the diluted sample.
3. The recommended dilution for specimen containing greater than 40 ng/mL is 1:5. However, it is desirable to dilute the samples that contain more than 40 ng PROG/mL so that the diluted sample reads between 2.0 and 20 ng PROG/mL.

Results

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

Limitations

The AIA-PACK PROG 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 Bioscience, Inc., South San Francisco, CA.

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