Alinity i Free PSA-11

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BIENNIAL REVIEW:

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SUPERSEDES: Procedure titled _____

CAUTION: United States Federal Law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to, by, or on the order of a physician.

Instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from these instructions.

WARNING: The concentration of free PSA (FPSA) in a given specimen, determined with assays from different manufacturers, can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the free PSA assay used. Values obtained with different assay methods cannot be used interchangeably. Note: % FPSA ratios must be calculated using Total PSA and Free PSA results both obtained on the Alinity i analyzer.

INTENDED USE

The Alinity i Free PSA assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of free prostate specific antigen (PSA) in human serum on the Alinity i analyzer.

The Alinity i Free PSA assay is intended to be used in conjunction with the Alinity i Total **PSA assay in men aged 50 years or older with total PSA values between 4 and 10 ng/mL** and DRE non-suspicious for cancer to determine the % free PSA value. The Alinity i % free PSA value can be used as an aid in discriminating between prostate cancer and benign disease.

SUMMARY AND EXPLANATION OF THE TEST

Prostate specific antigen (PSA), a member of the human kallikrein gene family, is a serine protease with chymotrypsin-like activity.*1*, *2*, *3* The mature form of PSA is a single chain glycoprotein of 237 amino acids containing 7-8% carbohydrate as a single N-linked oligosaccharide side chain. PSA has a molecular weight of approximately 30 000 daltons.*1*, *3*, *4*

The major site of PSA production is the glandular epithelium of the prostate. PSA produced by the prostate is secreted into the seminal fluid in high concentrations. PSA is also present in urine and serum.*3* The function of PSA is the proteolytic cleavage of gel forming proteins in the seminal fluid resulting in liquefaction of the seminal gel and increased sperm mobility.*3*, *5* Low levels of PSA are found in the blood as a result of leakage of PSA from the prostate gland. Increasing levels of PSA are associated with prostatic pathology; including prostatitis, benign prostatic hyperplasia (BPH), and cancer of the prostate.*6*, *7*, *8*, *9*

PSA occurs in three major forms in blood. The major immunodetectable form is PSA complexed with the serine protease inhibitor, alpha-1-antichymotrypsin (PSA-ACT). Uncomplexed, or free PSA, is the other immunodetectable form of PSA in serum. The majority of free PSA in serum appears to be an inactive form that cannot complex with protease inhibitors and may be either a PSA zymogen or an enzymatically-inactive, cleaved form of PSA. A third form of PSA, a complex with alpha-2-macroglobulin (AMG), is not detectable with current immunoassays for PSA due to the engulfment and subsequent masking of PSA epitopes by the alpha-2-macroglobulin molecule.*2*, *3*, *10*

Immunoassays have been designed to detect free PSA, PSA-ACT complex, and total PSA (immunodetectable forms: e.g., free PSA and PSA-ACT).*10*, *11*, *12* Using these types of assays, the proportion of free PSA in the serum was found to be significantly higher in patients with BPH than in patients with prostate cancer ($p < 0.00001$).*12* The proportion, or percent, of free PSA determined by comparing the concentration of free PSA to the concentration of total PSA has been proposed as a way to improve the discrimination between BPH and prostate cancer, especially in those men with intermediate levels of total serum PSA.*10*, *12*, *13*, *14*, *15*, *16*, *17*

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay is a two-step immunoassay for the quantitative determination of free PSA in human serum using chemiluminescent microparticle immunoassay (CMIA) technology.

Sample and anti-free PSA coated paramagnetic microparticles are combined and incubated. The free PSA present in the sample binds to the anti-free PSA coated microparticles. The mixture is washed. Anti-PSA acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of free PSA in the sample and the RLUs detected by the system optics.

For additional information on system and assay technology, **refer to the Alinity ci-series Operations Manual, Section 3.**

REAGENTS

Kit Contents

Alinity i Free PSA Reagent Kit 07P93

Volumes (mL) listed in the table below indicate the volume per cartridge.

REF	07P9321	07P9331
Tests per cartridge	100	500
Number of cartridges per kit	2	2
Tests per kit	200	1000
MICROPARTICLES	6.6 mL	27.0 mL
CONJUGATE	6.1 mL	26.5 mL

MICROPARTICLES Anti-Free PSA (mouse, monoclonal) coated Microparticles in TRIS buffer with protein (bovine) stabilizer. Minimum concentration: 0.1% solids. Preservatives: Antimicrobial Agents.

CONJUGATE Anti-PSA (mouse, monoclonal) acridinium-labeled Conjugate in MES buffer with protein (bovine) stabilizer. Minimum concentration: 10 ng/mL. Preservatives: Antimicrobial Agents.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use
- Rx ONLY

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[18], [19], [20], [21]

Safety Data Sheets are available at www.abbottdiagnostics.com or /and SDS folder.

For a detailed discussion of safety precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 8.**

Reagent Handling

Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facing down. This ensures that liquid covers all sides of the bottles within the cartridges. During reagent shipment, microparticles can settle on the reagent septum.

- Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.
- After mixing, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 7.**

Reagent Storage

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour before use.

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Onboard	System Temperature	30 days	
Opened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright during storage, discard the cartridge. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance.

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.

For information on unloading reagents**, refer to the Alinity ci-series Operations Manual, Section 5.**

Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when:

- a calibration error occurs
- a control value is out of the specified range

Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10.**

INSTRUMENT PROCEDURE

The Alinity i Free PSA assay file must be installed on the Alinity i analyzer prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 2.**

For information on printing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 5.**

For a detailed description of system procedures, refer to the Alinity ci-series Operations Manual.

Alternate Result Units

Edit assay parameter "Result Units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) x (Conversion factor) = (Concentration in Alternate result unit)

Default Result Unit	Conversion Factor	Alternate Result Unit
ng/mL	1.0	µg/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen type listed below was verified for use with this assay on the ARCHITECT i System.

Other specimen types and collection tube types have not been verified with this assay.

Specimen Type	Collection Tubes
Serum	Serum Serum separator

Specimen Conditions

Do not use:

- grossly hemolyzed specimens
- specimens with obvious microbial contamination

- For accurate results, serum specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- It is recommended to obtain specimens for PSA testing prior to procedures involving manipulation of the prostate.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer’s processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Insufficient processing of sample, or disruption of the sample during transportation may cause depressed results.

- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy may exhibit increased clotting time. If specimens are centrifuged before a complete clot forms, the presence of fibrin or particulate matter may cause erroneous results. Centrifuge specimens containing fibrin, red blood cells, or particulate matter. **Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.**
- If proper specimen collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter. Aliquots poured versus pipetted from specimen tube types that do not include serum separators are at higher risk of including particulates and generating depressed results.
- Failure to follow these instructions may result in depressed specimen results.
- Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

To ensure consistency in results, recentrifuge specimens prior to testing if

- they contain fibrin, red blood cells, or other particulate matter.

NOTE: If fibrin, red blood cells, or other particulate matter are observed, mix by low speed vortex or by inverting 10 times prior to recentrifugation.

Prepare frozen specimens as follows:

- Frozen specimens must be completely thawed before mixing.
- Mix thawed specimens thoroughly by low speed vortex or by inverting 10 times.
- Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous.
- If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Recentrifuge specimens.

Recentrifugation of Specimens

- Transfer specimens to a centrifuge tube and centrifuge from 50 000 to 100 000 g-minutes.
- Examples of acceptable time and force ranges that meet this criterion are listed in the table below.

  Centrifugation time using alternate RCF values can be calculated using the following formula:

$$\text{Centrifugation time (minutes)} = \frac{50\,000 \text{ g-minutes}}{\text{RCF}}$$

Centrifugation Time (Minutes)	RCF (x g)	g-Minutes
10	5000 - 10 000	50 000 - 100 000

$$RCF = 1.12 \times r_{max} (rpm/1000)^2$$

RCF -	The relative centrifugal force generated during centrifugation.
rpm -	The revolutions per minute of the rotor on which the specimens are being spun (usually the digital readout on the centrifuge will indicate the rpm).
Centrifugation Time -	The time should be measured from the time the rotor reaches the required RCF or rpm to the time it begins decelerating.
r_{max} -	Radius of the rotor in millimeters. NOTE: If custom tube adapters (i.e., adapters not defined by the centrifuge manufacturer) are used, then the radius (r_{max}) should be manually measured in millimeters and the RCF calculated.
g-minutes -	The unit of measure for the product of RCF (× g) and centrifugation time (minutes).

- Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.

Specimen Storage

Specimen storage conditions were verified on the ARCHITECT i System.

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Serum	2 to 8°C	24 hours	Serum should be separated from the clot within 3 hours from time of collection and stored at 2-8°C for up to 24 hours.

The serum, if not tested within 24 hours, should be frozen at -20°C or colder.[22], [23]

Avoid multiple freeze/thaw cycles.

Specimen Shipping

Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.

Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

07P93 Alinity i Free PSA Reagent Kit

Materials Required but not Provided

- Alinity i Free PSA assay file
- 07P9301 Alinity i Free PSA Calibrators
- 07P9310 Alinity i Free PSA Controls or other commercially available controls
- Alinity Trigger Solution
- Alinity Pre-Trigger Solution
- Alinity i-series Concentrated Wash Buffer

For information on materials required for operation of the instrument, **refer to the Alinity ci-series Operations Manual, Section 1.**

For information on materials required for maintenance procedures, **refer to the Alinity ci-series Operations Manual, Section 9.**

Assay Procedure

For a detailed description of how to run an assay, **refer to the Alinity ci-series Operations Manual, Section 5.**

- If using primary or aliquot tubes, refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.
- To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

Maximum number of replicates sampled from the same sample cup: 10

Priority:

- Sample volume for first test: 140 µL
- Sample volume for each additional test from same sample cup: 90 µL

≤ 3 hours on the reagent and sample manager:

- Sample volume for first test: 150 µL
- Sample volume for each additional test from same sample cup: 90 µL

> 3 hours on the reagent and sample manager:

    - Replace with a fresh aliquot of sample.

- Refer to the Alinity i Free PSA calibrator package insert and/or Alinity i Free PSA control package insert for preparation and usage.
- For general operating procedures, **refer to the Alinity ci-series Operations Manual, Section 5.**
- For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, **Section 9. Perform maintenance more frequently when required by laboratory procedures.**

Sample Dilution Procedures

Samples cannot be diluted for the Alinity i Free PSA assay. Samples with a free PSA concentration of > 30 ng/mL (> 30 µg/L) are flagged as > 30.00 ng/mL (> 30.00 µg/L) and need to be reported as such.

Calibration

For instructions on performing a calibration, **refer to the Alinity ci-series Operations Manual, Section 5.**

Each assay control must be tested to evaluate the assay calibration.

Once a calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:

- A reagent kit with a new lot number is used.
- Controls are out of range.

Quality Control Procedures

The recommended control requirement for the Alinity i Free PSA assay is that a single sample of each control level be tested once every day testing performed.

Verification of Assay Claims

For protocols to verify package insert claims, refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.

RESULTS

Calculation

The Alinity i Free PSA assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration and results.

Calculation of Alinity i % Free PSA Value

The Alinity i % Free PSA Value can be calculated when both Alinity i Free PSA and Alinity i Total PSA results are obtained for the same sample.

The Alinity i analyzer can automatically calculate a % free PSA value.

% free PSA = (Alinity i Free PSA / Alinity i Total PSA) x 100

For information on alternate result units, refer to the INSTRUMENT PROCEDURE, Alternate Result Units section of this package insert.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the Alinity ci-series Operations Manual, Section 5.

Measuring Interval

Measuring interval is defined as the range of values in ng/mL (µg/L) which meets the limits of acceptable performance for linearity, imprecision, and bias.

The measuring interval of the Alinity i Free PSA assay is **0.1 to 30.0 ng/mL** (0.1 to 30.0 µg/L).

LIMITATIONS OF THE PROCEDURE

- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies.[24, 25] Alinity i Free PSA reagents contain a component that reduces the effect of HAMA reactive specimens. Additional clinical or diagnostic information may be required to determine patient status.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed. Additional information may be required for diagnosis.[26]
- The concentration of PSA in a given specimen, determined with assays from different manufacturers, can vary due to differences in assay methods, calibration, and reagent specificity.[3, 27, 28]
- Quality control samples may be produced by introducing seminal fluid PSA into a human serum matrix. PSA in serum and seminal fluid may exist in different forms. The concentration of PSA in these controls, determined with assays from different manufacturers, can vary due to differences in assay methods, calibration, reagent specificity, and the form of PSA that is present; therefore, it is important to use assay-specific values to evaluate control results.
- Digital rectal examination (DRE) may cause clinically significant changes in the free PSA and free/total PSA ratio in some patients.[29] Additionally, prostatic massage, ultrasonography, cystoscopy, and needle biopsy may cause clinically significant elevations.[29, 30] Serum for free PSA determinations should be drawn before performing prostatic manipulations. PSA levels may also be increased following ejaculation.[31]

- Active free PSA in the serum at the time of blood sampling, can continue to complex with serum protease inhibitors, especially alpha-2-macroglobulin, resulting in a rapid decrease in PSA levels of the active form of free PSA.[32]
- Hormonal therapy may affect PSA expression; therefore, a low PSA level after any treatment that includes hormonal therapy may not adequately reflect the presence of residual or recurrent disease.[33]
- The measurement of free PSA or the free/total PSA ratio is not an absolute test for malignancy. The PSA values should be used in conjunction with information available from the clinical evaluation and other diagnostic procedures: *e.g.* symptoms, clinical impressions, digital rectal examination, transrectal ultrasound, etc. A prostatic biopsy is required for the diagnosis of cancer.

EXPECTED VALUES

These studies were performed on the ARCHITECT i System.

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

It is recommended that each laboratory determine its own reference range based upon its particular locale and population characteristics.

The distribution of ARCHITECT Free PSA values determined in apparently healthy males, males with BPH, and males with stage A and B prostate cancer is shown below.

Distribution of ARCHITECT Free PSA Values

		Percent (%)				
	Number of Subjects	**0-0.5 (ng/mL)**	**> 0.5-2.5 (ng/mL)**	**> 2.5-5.0 (ng/mL)**	**> 5.0-10 (ng/mL)**	**> 10 (ng/mL)**
Healthy Males	475	87.2	12.8	0.0	0.0	0.0
BPH	212	51.9	42.9	4.2	0.5	0.5
Stage A Prostate Cancer	26	38.5	42.3	11.5	3.8	3.8
Stage B Prostate Cancer	67	23.9	68.7	7.5	0.0	0.0

The distribution table is derived from 475 apparently healthy male subjects with no clinical evidence of prostate cancer, 212 males with BPH, and 93 males with active prostate cancer.

A prospective study of 430 subjects was conducted at nine clinical sites. A fixed cutoff of 26% was used to determine the sensitivity and specificity for subjects with a total PSA range of 4 to 10 ng/mL and a DRE non-suspicious for cancer. The total and free PSA values were determined using the ARCHITECT Free PSA and ARCHITECT Total PSA assays. At the fixed cutoff of 26%, the ARCHITECT Free PSA assay yielded a sensitivity of 91.1% and a specificity of 18.2%.

The distribution of ARCHITECT % free PSA values was determined for the same 430 subjects (307 biopsy negative and 123 biopsy positive). The % free PSA values were divided into five groups by the following boundaries: ≤ 10, > 10-15, > 15-20, > 20-26, and > 26. The table below shows the % free PSA values.

Distribution of ARCHITECT % Free PSA Values for Specimens with ARCHITECT Total PSA Between 4 and 10 ng/mL

	Distribution of Subjects (%)					
	Number of Subjects	% Free PSA Ranges				
		≤ 10	> 10-15	> 15-20	> 20-26	> 26
Biopsy Negative	307	9.4	22.5	25.4	24.8	17.9
Biopsy Positive	123	27.6	30.9	17.9	15.4	8.1

The probabilities of prostate cancer given the value in specific ranges for % free PSA were calculated based on a logistic regression model using the same group of subjects as above. Prostate cancer probabilities associated with % free PSA values are dependent on the disease prevalence within the study population.[34] In this study, the probabilities of prostate cancer are representative of a patient population for both screening and referral site with an overall disease prevalence of approximately 29%.[35] The table below shows the distribution of cancer probabilities of % free PSA using the same study population adjusted for different rates of disease prevalence.

Probability of Prostate Cancer by Disease Prevalence for Subjects with ARCHITECT Total PSA Between 4 and 10 ng/mL and DRE Non-suspicious for Cancer

Disease Prevalence Rate (%)	% Free PSA Ranges				
	≤ 10	> 10-15	> 15-20	> 20-26	> 26
25[36]	44.0	32.9	23.4	16.0	10.6
29	48.6	37.1	26.9	18.6	12.5
35	56.0	44.2	33.1	23.5	16.1

The estimates of cancer probability may be influenced by the presence of other risk factors.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity i analyzer and the ARCHITECT i System utilize the same reagents and sample/reagent ratios.

Unless otherwise specified, all studies were performed on the Alinity i analyzer.

Precision

Within-Laboratory Precision

Studies were performed based on guidance from CLSI EP05-A2.[37] Each sample was tested using 3 lots of the Alinity i Free PSA Reagent Kit, 3 lots of the Alinity i Free PSA Calibrators, and 3 lots of the Alinity i Free PSA Controls and 1 instrument. The 3 controls were assayed in 3 replicates at 2 separate times per day on 20 different days. The 6 native male serum-based panels were assayed in 2 replicates at 2 separate times per day on 20 different days.

			Mean ng/mL	Within-Run		Within-Laboratory (Total)[a]	
Sample	**Lot**	**n**	**(µg/L)**	**SD**	**%CV**	**SD**	**%CV**
Low Control	1	119	0.393	0.0147	3.7	0.0175	4.5
	2	120	0.394	0.0112	2.8	0.0145	3.7
	3	120	0.418	0.0107	2.6	0.0140	3.3
Medium Control	1	120	0.991	0.0252	2.5	0.0384	3.9
	2	119	0.990	0.0261	2.6	0.0341	3.4
	3	119	1.046	0.0256	2.4	0.0303	2.9
High Control	1	120	6.817	0.2143	3.1	0.3145	4.6
	2	119	6.833	0.1889	2.8	0.2360	3.5
	3	120	7.268	0.2366	3.3	0.3000	4.1
Panel 1	1	80	0.092	0.0024	2.6	0.0035	3.8
	2	80	0.100	0.0036	3.6	0.0042	4.1
	3	80	0.095	0.0031	3.3	0.0041	4.3
Panel 2	1	80	0.957	0.0234	2.4	0.0250	2.6
	2	80	1.016	0.0302	3.0	0.0318	3.1
	3	80	0.996	0.0289	2.9	0.0306	3.1
Panel 3	1	80	2.575	0.0563	2.2	0.0715	2.8
	2	80	2.735	0.0739	2.7	0.0896	3.3
	3	80	2.659	0.0785	3.0	0.0916	3.4
Panel 4	1	80	8.610	0.3490	4.1	0.3490	4.1

Sample	Lot	n	Mean ng/mL (µg/L)	Within-Run		Within-Laboratory (Total)[a]	
				SD	%CV	SD	%CV
	2	80	9.175	0.2968	3.2	0.3384	3.7
	3	80	8.710	0.3094	3.6	0.3958	4.5
Panel 5	1	80	23.190	0.7604	3.3	0.9963	4.3
	2	80	25.315	0.8983	3.5	0.9252	3.7
	3	80	23.605	0.8049	3.4	0.9304	3.9
Panel 6	1	80	0.207	0.0072	3.5	0.0073	3.5
	2	80	0.223	0.0067	3.0	0.0076	3.4
	3	80	0.214	0.0078	3.6	0.0079	3.7

[a]Includes within-run, between-run, and between-day variability.

System Reproducibility

A study was performed based on guidance from CLSI EP05-A2 and CLSI EP15-A2.*37*, *38* Testing was conducted at 3 clinical sites using 1 lot each of the Alinity i Free PSA Reagent Kit, Alinity i Free PSA Calibrators, and Alinity i Free PSA Controls and 1 instrument per site. Three controls and 5 native male serum-based panels were assayed in replicates of 4 at 2 separate times per day for 5 different days.

Sample	n	Grand Mean ng/mL (µg/L)	Within-Run		Within-Day[a]		Within-Laboratory Precision (Total)[b]		Precision with Additional Component of Between-Site (Overall)	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Control	120	0.418	0.0104	2.5	0.0104	2.5	0.0106	2.5	0.0144	3.4
Medium Control	120	1.042	0.0216	2.1	0.0245	2.4	0.0249	2.4	0.0294	2.8
High Control	120	7.066	0.1727	2.4	0.1890	2.7	0.1890	2.7	0.2463	3.5
Panel 1	120	0.205	0.0063	3.1	0.0063	3.1	0.0068	3.3	0.0070	3.4
Panel 2	120	0.922	0.0293	3.2	0.0293	3.2	0.0297	3.2	0.0317	3.4

Sample	n	Grand Mean ng/mL (µg/L)	Within-Run		Within-Day[a]		Within-Laboratory Precision (Total)[b]		Precision with Additional Component of Between-Site (Overall)	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Panel 3	120	2.488	0.0693	2.8	0.0693	2.8	0.0693	2.8	0.0784	3.2
Panel 4	120	8.345	0.2853	3.4	0.2856	3.4	0.2856	3.4	0.3296	3.9
Panel 5	120	22.597	0.7733	3.4	0.7761	3.4	0.7852	3.5	1.0308	4.6

[a] Includes within-run and between-run variability.

[b]Includes within-run, between-run, and between-day variability.

Lower Limits of Measurement

A study was performed based on guidance from CLSI EP17-A2.*39* Testing was conducted using 3 lots of the Alinity i Free PSA Reagent Kit on each of 2 instruments over a minimum of 3 days. The maximum observed Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) values are summarized below.

	ng/mL	µg/L
LoB[a]	0.000	0.000
LoD[b]	0.002	0.002
LoQ[c,d]	**0.100**	0.100

[a] The LoB represents the 95th percentile from n ≥ 60 replicates of zero-analyte samples.

[b] The LoD represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples.

[c] The LoQ is defined as the lowest concentration at which the maximum allowable precision of 15 %CV was met and was determined from n ≥ 60 replicates of low-analyte level samples.

[d]This value represents the observed LoQ on the ARCHITECT System. The LoQ observed on the Alinity i analyzer supports this LoQ.

Linearity

A study was performed based on guidance from CLSI EP06-A.*40*

This assay is linear across the measuring interval of **0.1 to 30.0 ng/mL** (0.1 to 30.0 µg/L).

Comparison Between the Alinity i Analyzer and the ARCHITECT i2000SR System

The comparison between the Alinity i analyzer and the ARCHITECT i2000SR system was evaluated with serum sample results using 1 lot each of the Alinity i Free PSA Reagent Kit,

Alinity i Free PSA Calibrators, and Alinity i Free PSA Controls. Testing of each sample was performed on 1 Alinity i analyzer at each of 3 clinical testing sites and on 1 ARCHITECT i2000SR instrument at 1 clinical testing site. A Deming regression was performed using the results from each of the 3 Alinity i clinical testing sites versus the ARCHITECT results from 1 clinical testing site.

		Concentration Range ng/mL (µg/L)		Correlation Coefficient (r)					
Clinical Site	**N**	**Alinity i**	**ARCHITECT i2000SR**	**r**	**95% CI[a]**	**Intercept**	**95% CI[a]**	**Slope**	**95% CI[a]**
1	188	0.102 - 27.977	0.113 - 28.734	0.998	(0.998, 0.999)	-0.01	(-0.01, -0.01)	0.98	(0.97, 0.98)
2	189	0.101 - 29.987	0.113 - 29.018	0.998	(0.997, 0.998)	-0.01	(-0.02, -0.01)	0.97	(0.96, 0.98)
3	190	0.103 - 28.034	0.113 - 29.018	0.998	(0.997, 0.998)	-0.00	(-0.01, -0.00)	0.92	(0.91, 0.93)

[a]95% CI = Confidence Interval

Carryover

No significant carryover (≤ 0.05 ng/mL) was observed when a sample containing 7208.65 ng/mL of free PSA was assayed.

Analytical Specificity

This study was performed on the ARCHITECT i System.

The specificity of the ARCHITECT Free PSA assay was determined by testing sera containing the following compounds. These compounds showed less than or equal to 10% interference in the ARCHITECT Free PSA assay at the levels indicated.

Interfering Substances

Test Compound	Concentration
Bilirubin	20 mg/dL
Hemoglobin	500 mg/dL
Total Protein	2.0 g/dL & 12.0 g/dL
Prostatic Acid Phosphatase	1000 ng/mL
Triglycerides	3000 mg/dL
Hytrin	20 µg/mL
Proscar	25 µg/mL
Flomax	1 µg/mL

High Dose Hook

This study was performed on the ARCHITECT i System.

High dose hook is a phenomenon whereby very high level specimens may read within the dynamic range of the assay. For the ARCHITECT Free PSA assay, no high dose hook effect was observed when samples containing up to 2400 ng/mL of free PSA were assayed.

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