Instructions for Use

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Sangia Total PSA Test (PN 40025)

Indication for Use:

The Sangia Total PSA Test is an immunoassay indicated to quantitatively measure Total PSA in capillary whole blood from a fingerstick collected by a healthcare professional and is used in conjunction with a digital rectal exam (DRE) as an aid in the detection of prostate cancer in men aged 50 years and older. The Sangia Total PSA Test is performed using the Claros 1 Analyzer in point-of-care settings. A prostate biopsy is required for the diagnosis of prostate cancer.

Contraindications:

There are no known contraindications.

Warning:

This device is not intended for the serial measurement of total PSA to aid in the management of prostate cancer patients.

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.

Summary and Explanation

Prostate Specific Antigen (PSA) is a member of the human kallikrein family of serine proteases with chymotrypsin-like activity.[1] PSA is a major protein in seminal fluid secreted by prostate epithelial cells,[2] with a primary physiological role of semen liquefaction.[3] PSA has also been detected in other tissues and body fluids of both males and females.[4] The proteolytic activity of PSA is modulated by polypeptide bond cleavage[5,6,7] or the irreversible formation of complexes with protease inhibitors such as α_1-antichymotrypsin (ACT), α_2-macroglobulin (A2M) and other acute phase proteins.[5] There are three major groups of PSA proteins in blood.[8] Circulating PSA complexed with A2M is completely engulfed and inaccessible to detection by immunoassay. The two detectable forms of PSA (collectively called Total PSA) are PSA complexed with ACT (complexed PSA or PSA-ACT), and uncomplexed or Free PSA. The Sangia Total PSA Test is an equimolar assay, meaning it equally detects PSA-ACT and Free PSA. Total PSA is present in small quantities in the serum of men with healthy prostates and is elevated in the presence of prostate cancer.[9,10] Other prostate disorders such as benign prostate hyperplasia (BPH) or prostatitis can also cause elevated Total PSA.[11] Stimulation or trauma of the prostate gland (e.g., ejaculation, biopsy, cystoscopy, transurethral resection of prostate, and radical prostatectomy)[12,13,14] transiently increase Total PSA. Drug therapy with 5α-reductase inhibitors (5-ARIs), used primarily in the treatment of BPH, will lower Total PSA.[15]

Test Principle

The Sangia Total PSA Test is a sandwich immunoassay based on Silver Amplified NeoGold ImmunoAssay (Sangia®) technology performed in a microfluidic cassette. The Sangia Total PSA Test is used in conjunction with the Claros®1 Analyzer. All reagents necessary to carry out a Total PSA test are contained in the consumables that include a Cassette Assembly and a Sample Collector.

Fingerstick blood is collected into the Sample Collector via capillary action. The Sample Collector contains lyophilized reagents (including anti-PSA monoclonal antibodies labeled with NeoGold), which are dissolved by the blood. In the reconstituted mixture, Total PSA in the blood sample binds to NeoGold-labeled anti-PSA antibodies to form labeled antigen-antibody complexes.

The user connects the Sample Collector to the Cassette Assembly and the test cassette is inserted into the Claros 1 Analyzer. Using the analyzer touch screen, the user can enter patient information and initiate the test. All assay steps outlined below are performed automatically by the analyzer, which will provide the test result within approximately 10-12 minutes:

- Sample incubation: The analyzer applies a vacuum to the test cassette, pulling the sample mixture from the Sample Collector into a microfluidic portion of the test cassette. The sample flows through multiple measurement zones, including a test zone, a negative control zone, and a positive control zone. In the test zone, the labeled complexes bind to a second set of anti-PSA antibodies attached to the surface of the channel walls, forming an antibody-antigen-antibody sandwich.
- Removal of unbound materials: A sequence of automatic wash steps removes sample components and reagents that are not specifically bound to capture antibodies in the measurement zones.
- Amplification and detection of signal: A silver amplification reagent automatically flows over all measurement zones, reacting with all available NeoGold. The reaction results in the deposition of a silver metallic film within the measurement zone that interferes with light transmission. The optical density of this film is a function of the concentration of Total PSA in the sample.
- Internal controls: Similar reactions in the negative and positive control zones are used to ensure proper assay validity. If these controls are outside the expected ranges, the test is automatically invalidated and a result is not reported.
- Result output: The Sangia Total PSA Test is factory calibrated, and the calibration information is included on a Lot Data Card provided in each box of tests. The Total PSA concentration is reported (ng/mL) based on the optical readings and calibration information stored in the analyzer. Valid test results are displayed and stored on the analyzer.

Test Composition

Each Sangia Total PSA Test box contains:

- (20) Sangia Total PSA Tests stored in individually sealed pouches (PN 40025)
- (1) Lot Data Card with lot-specific calibration data
- (23) Soap Wipes, which contain water and sodium lauryl sulfate
- (1) Package Insert (Instructions for Use)

Each Sangia Total PSA Test (PN 40025) includes:

- (1) Test Cassette, which contains trace amounts of monoclonal anti-PSA antibodies (bound to the surface of the measurement zone), aqueous solution of silver salt (45µL), aqueous solution of reducing agent in acidic buffer (45µL), aqueous wash buffers with surfactants (<5µL), protein stabilizers and blockers, and preservatives.
- (1) Sample Collector, which contains NeoGold-labeled anti-PSA antibodies, anticoagulant, buffers, protein stabilizers, and surfactants in a lyophilized formulation.

Materials Required but not Provided

Claros 1 Analyzer (PN 40001)

Claros Total PSA External Controls (PN 40146 and 40147)

The Sangia Total PSA Test requires the following disposables (not included with the Test):

- Exam gloves
- Alcohol swab
- Gauze pad
- Sterile, single use lancet (sufficient to collect at least 20µL of blood)
- Bandage

Limitations and Precautions

1. For diagnostic purposes only: the Sangia Total PSA Test results should always be assessed in conjunction with the patient's medical history, clinical examination, and other findings.
2. Testing of capillary whole blood from a fingerstick in point-of-care settings can lead to increased imprecision of test results. Physicians should confirm results near the clinical decision point(s) with a clinical laboratory test.
3. Samples obtained from patients immediately following DRE, prostatic massage, or ultrasonography of the prostate could affect PSA levels.[17,18] Invasive procedures such as needle biopsy, transurethral resection of prostate, and cystoscopy may cause persistent, clinically significant elevations in Total PSA levels.[13] Total PSA levels may also be increased following ejaculation.[12]
4. Samples from patients who have received preparations of mouse monoclonal antibodies for therapy or diagnostic procedures may contain human anti-mouse antibodies (HAMA). Such samples may show either falsely elevated or depressed values when tested with assays which employ mouse monoclonal antibodies.[16]
5. The Cassette Assembly contains trace amounts hazardous materials, including silver nitrate and sodium azide. These materials are entirely contained within the Cassette Assembly and are in quantities and concentrations that do not pose a hazard.
6. The used test cassette should be disposed of in an appropriate biohazard waste container.
7. Each Sangia Total PSA Test is a single use device, do not attempt to re-use a Sangia Total PSA Test.
8. Do not use the Sangia Total PSA Test if the packaging, container, or test components appear damaged (broken, crushed, leaking, etc.).

Storage

Sangia Total PSA Tests should be stored prior to use at 2°C to 8°C until the expiration date.

Do not freeze the Sangia Total PSA Tests.

Specimen

Capillary whole blood obtained from a fingerstick. Use standard personal protective equipment to collect the sample and conduct the test according to the policies of your organization (such as lab coat, exam gloves, eye protection).

Calibration

Each lot of Sangia Total PSA Tests is calibrated at the time of manufacturing and calibration information is recorded on a Lot Data Card provided with each Sangia Total PSA Test box. No user calibration of tests is required.

For each new lot of Sangia Total PSA Tests to be used, the user must upload the calibration data for the lot into the Claros 1 Analyzer from the Lot Data Card according to the *Claros 1 System Operation Manual*.

The Claros 1 Analyzer can store lot information for the most recent 100 lots uploaded by the user.

Test Procedure

See the ***Quick Reference Guide*** for a summary of the Test Procedure.

See the ***Claros 1 System Operation Manual*** for a detailed explanation of all Claros 1 Analyzer functions.

1. Ensure the Claros 1 Analyzer is on and displaying the "**Ready**" screen.
2. Remove a **Sangia Total PSA Test** pouch from storage and gather the needed **Disposables**.
3. Open the Test pouch and remove the Sample Collector and Test Cassette. Remove the strip of foil from the Cassette.
4. **Prepare the patient's sample site** to promote good blood flow. Have the patient wash hands in warm water, and if needed, gently massage the finger and confirm the fingers are warm.
5. **Identify the patient sample site**, on the outer edge toward the tip of the middle or ring finger.
6. Clean the finger site thoroughly for about 5 seconds with the **soap wipe** (provided with the tests).
7. Clean and remove any remaining soap from the finger site with an **alcohol swab**. Allow the finger to air dry completely for about 10 seconds.
8. Perform the fingerstick, turn the finger downward, and firmly squeeze a drop of blood **without "milking"** the finger.
9. Turn the finger downward and express and **wipe off the first two or more large drops of blood** with a gauze pad.
10. With the finger pointing downward, firmly squeeze the finger again to obtain another large hanging drop of blood.
11. Scoop the hanging drop of blood into the end of the Sample Collector. The blood will be drawn into the capillary tube of the Sample Holder and must **completely fill the tube**. Cover the patient's finger with the gauze or apply a bandage if needed.
12. Remove the Collector and discard it in a biohazard container. **Insert the Sample Holder** completely into the guide of the Cassette, pressing evenly on each side with both hands, so that it snaps securely in place.
13. **Insert the Test Cassette** into the front of the Claros 1 Analyzer and follow the instructions on the screen to start the test. Start the test as soon as possible after opening the Test and collecting the blood sample
14. When the test is complete, within approximately 10-12 minutes, the Claros 1 Analyzer will display the **test result** on the screen.
15. Remove the used Test Cassette from the Claros 1 Analyzer and **dispose** of it in a biohazard container.

Quality Control Procedure

The Claros Total PSA External Controls Level 1 and Level 2 are the only external controls for use with the Sangia Total PSA Test.

Run and pass external controls according to your organization's Quality Control policies. To run external controls, follow the instructions in the *Claros 1 System Operation Manual* or the *Quick Reference Guide*.

Calculation

The test result is provided automatically by the analyzer and no user calculation is required.

Traceability of Calibrators

The assay is calibrated with calibrators containing a mixture of PSA-ACT and Free PSA (90:10 ratio), the calibrators are traceable to the WHO standard (NIBSC 96/670).

Performance Characteristics

Measuring Interval

The Sangia Total PSA Test measuring interval is between 0.08 ng/mL and 15 ng/mL. Values below 0.08 ng/mL are reported as <0.08 ng/mL. Values above 15 ng/mL are reported as >15 ng/mL. No dilutions are allowed.

Expected Values/Reference Interval

The distribution of Sangia Total PSA Test results was evaluated in a cohort of 430 apparently healthy men aged 50 years or older, from 13 U.S. sites. The mean age was 64.3 (95% CI 45.7-83.0), and the median age was 62.6. Using a cutoff of 4.0 ng/mL, 97% of apparently healthy men aged 50 to 60 years of age had Sangia Total PSA values less than 4.0 ng/mL.

Age	N	Sangia Total PSA (ng/mL)		
		95th percentile (95% CI)	Median (25th percentile, 75th percentile)	% subjects with PSA <4 ng/mL
50-59	167	3.5 (2.4-4.9)	0.86 (0.51, 1.6)	97.0%
60-69	142	5.3 (4.3-7.8)	1.45 (0.64, 2.9)	88.0%
70+	121	7.8 (5.4-11.7)	1.50 (0.77, 3.0)	83.5%

Each laboratory should establish its own expected values/reference intervals with its own patient population.

Clinical Performance

A prospective clinical study was conducted at 10 urology practices in the U.S. to demonstrate the clinical validity of the Sangia Total PSA Test in conjunction with DRE. The cohort was composed of 434 men who were aged 50 or older and scheduled to receive a prostate biopsy. Men were excluded if they had any invasive urologic procedure or were taking a 5-alpha-reductase inhibitor (5 -ARI) medication in the 3 months leading up to the testing, or if they had a DRE performed within four days prior to testing.

The prevalence of prostate cancer in the cohort was 53.7% (233/434), and the median age of the subjects was 65.1 years old. The cohort was primarily Caucasian (85.0%) and included 7.6% African American, 5.1% Hispanic and 2.3% of subjects of other and unknown origin. One hundred and seven men (25%) were reported to have abnormal DRE, and 339 men (78%) were found to have a Sangia Total PSA greater than 4.0 ng/mL.

The median Total PSA concentration in the cohort was 5.9 ng/mL. In the group of subjects that was biopsy positive (prostate cancer), the median Total PSA concentration was 6.3 ng/mL, and 5.0 ng/mL in the biopsy negative group (without prostate cancer).

The Sangia Total PSA Test results are summarized in the following tables:

All Patients with Prostate Cancer	Abnormal DRE	Normal DRE	Total
Sangia Total PSA ≥ 4 ng/mL	61	138	199
Sangia Total PSA < 4 ng/mL	13	21	34
Total	74	159	233

All Patients without Prostate Cancer	Abnormal DRE	Normal DRE	Total
Sangia Total PSA ≥ 4 ng/mL	16	124	140
Sangia Total PSA < 4 ng/mL	17	44	61
Total	33	168	201

The study data were analyzed to calculate the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for DRE and Total PSA with a cutoff of 4.0 ng/mL. The sensitivity of the Sangia Total PSA Test is 85.4% (199/233) with 95% CI: (80.3–89.4%) and specificity is 30.3% (61/201) with 95% CI: (24.4-37.0%). Data of the clinical study showed that the Sangia Total PSA Test is an informative test with regard to risks of prostate cancer. The sensitivity of the Sangia Total PSA Test used in conjunction with DRE is 91.0% (212/233, 95% CI: 86.6-94.3%), and the two tests used in combination detect 2.9 times (212/74) more cancers than DRE alone.

Test	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
DRE+	31.8% (74/233) (26.1-38.0%)	83.6% (168/201) (77.8-88.1%)	69.2% (74/107) (60.7-76.5%)	51.4% (168/327) (45.7-54.1%)
Sangia Total PSA (cutoff: 4.0 ng/mL)	85.4% (199/233) (80.3-89.4%)	30.3% (61/201) (24.4-37.0%)	58.7% (199/339) (56.2-61.4%)	64.2% (61/95) (55.2-72.5%)
Sangia Total PSA (cutoff: 4.0 ng/mL) or DRE+	91.0% (212/233) (86.6-94.0%)	21.9% (44/201) (16.7-28.1%)	57.5% (212/369) (55.4-59.7%)	67.7% (44/65) (56.2-77.3%)

Analytical Sensitivity

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined using K_2EDTA venous whole blood samples in accordance to CLSI EP17-A2 guideline.

The LoB was calculated from 144 measurements obtained with duplicates of four blank samples over three days on three Claros1 Analyzers using two Sangia Total PSA Test lots. The LoB was determined as 0.017 ng/mL.

The LoD was calculated from 144 measurements obtained with triplicates of four samples with low-levels of endogenous total PSA over three days on two Claros 1 Analyzers on two Sangia Total PSA Test lots. The LoD was determined as 0.027 ng/mL.

The LoQ was calculated from 504 measurements obtained with triplicates of seven samples with low-levels of endogenous total PSA over three days on four Claros 1 Analyzers on two Sangia Total PSA Test lots. The LoQ with K_2EDTA venous whole blood samples was determined as 0.035 ng/mL meeting the within-laboratory %CV of 15%. In addition, the LoQ with fingerstick whole blood samples was determined as 0.08 ng/mL meeting the within-laboratory %CV of 20%.

Precision

Precision using fingerstick whole blood samples was estimated in:

a. A single site study that tested 8 subjects in morning and afternoon sessions with 4 Sangia Total PSA tests performed from fingerstick capillary whole blood during each session for each subject yielded the following results:

Mean Sangia Total PSA (ng/mL)	N	SD	%CV
0.37	8	0.06	15.8%
0.41	8	0.05	11.2%
1.28	8	0.14	10.9%
1.54	8	0.11	6.9%
2.94	8	0.33	11.2%
5.13	8	0.71	13.9%
5.20	8	0.48	9.3%
12.87	6	1.18	9.2%

b. A multi-center study that tested 61 subjects with Sangia Total PSA values within the measuring interval. At each site, each subject was tested with the Sangia Total PSA Test by three operators, each operator collected the fingerstick sample from different fingers from the same subject. The study yielded the following results:

Group [Range of PSA concentration]		N	Mean (ng/mL)	SD	%CV
Very Low	[0.08 – 0.6 ng/mL]	6	0.25	0.04	17.8%
Low	[0.6 – 3.0 ng/mL]	11	1.91	0.22	11.6%
Medium	[3.0 – 6.0 ng/mL]	18	4.74	0.70	14.8%
High	[6.0 – 10.0 ng/mL]	18	8.04	0.84	10.5%
Very High	[10.0 – 15.0 ng/mL]	8	11.88	1.66	13.9%
Total		**61**			

Precision using artificial samples was estimated using the Single-Site study protocol (20 days x 2 runs x 2 replicates = 80 determinations per level), as described in CLSI EP05-A3.

Level	Mean Sangia Total PSA (ng/mL)	N	Within-Run (Repeatability)		Between-Run		Between-Day		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.16	80	0.02	9%	0.00	2%	0.01	6%	0.02	11%
2	0.29	80	0.03	10%	0.01	3%	0.01	2%	0.03	11%
3	0.94	80	0.09	9%	0.04	4%	0.04	4%	0.10	11%
4	3.06	80	0.29	9%	0.16	5%	0.00	0%	0.33	11%
5	4.43	80	0.45	10%	0.18	4%	0.20	4%	0.53	12%
6	13.81	80	0.90	7%	0.73	5%	0.73	5%	1.37	10%

Reproducibility

A multi-center study tested 8 K_2EDTA samples across the measuring range of the Sangia Total PSA Test. Six users at three sites assayed seven levels of total PSA on three unique lots of Sangia Total PSA Tests. Each level of total PSA was assayed 144 times (3 sites x 2 days x 2 runs/day x 3 lots of Tests x 2 Analyzers x 2 replicates = 144).

Mean Sangia Total PSA (ng/mL)	N	Within-Run (Repeatability)		Between-Run		Between-Lot		Between-Site		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
0.46	144	0.05	11%	0.01	2%	0.05	10%	0.02	5%	0.08	16%
0.57	144	0.06	10%	0.01	2%	0.06	10%	0.00	0%	0.09	15%
1.48	144	0.16	11%	0.03	2%	0.12	8%	0.00	0%	0.21	14%
2.53	144	0.28	11%	0.00	0%	0.26	10%	0.05	2%	0.38	15%
4.63	144	0.40	9%	0.00	0%	0.57	12%	0.00	0%	0.71	15%
6.71	144	0.65	10%	0.39	6%	0.79	12%	0.06	0%	1.10	16%
8.88	144	0.96	11%	0.27	3%	0.91	10%	0.24	3%	1.37	15%
12.38	144	1.38	11%	0.38	3%	1.00	8%	0.18	1%	1.78	14%

Method Comparison

A multi-center study tested 122 prospectively enrolled male subjects and compared the Sangia Total PSA Test results with an FDA-approved total PSA test results obtained from matched serum samples using Passing-Bablok regression analysis. The sample concentrations were between 0.1 and 13.1 ng/mL. The results are:

N	Slope (95% CI)	Intercept (95% CI)	Correlation
122	0.995 (0.940 to 1.073)	-0.011 (-0.091 to 0.093)	0.951

The systematic differences between the Sangia Total PSA Test results and the FDA-approved total PSA test results at the medical decision level (MDL) are presented below:

Systematic differences			
Total PSA Concentration	Systematic difference	%Systematic difference	95% CI
2.5	-0.023	-0.9%	(-4.9%; 5.8%)
4.0	-0.031	-0.8%	(-5.0%; 6.4%)
10.0	-0.061	-0.6%	(-5.4%; 6.8%)

Matrix Comparison

A multi-center study tested prospectively enrolled male subjects and compared the Sangia Total PSA results obtained from capillary fingerstick whole blood samples with the Sangia Total PSA results obtained from K_2EDTA anticoagulated whole blood samples using Passing-Bablok regression analysis. The concentrations of a total of 127 samples were between 0.08 and 14.9 ng/mL. The results of the regression analysis were: slope=1.091 with 95% CI: (1.030; 1.143) and intercept =-0.045 with 95% CI: (-0.113; 0.020); correlation=0.976.

Interference/Analytical Specificity

Interference studies were performed according to the recommendations of CLSI protocol EP07-A3 at total PSA concentrations of 0.3, 3.0 and 10.0 ng/mL. Hemoglobin (up to 500 mg/dL), triglycerides (up to 3.2 g/dL), unconjugated bilirubin (up to 20 mg/dL) and rheumatoid factor (up to 150 IU/mL) did not interfere with Sangia Total PSA Test. Added human IgG (up to 2.5 g/dL) and human serum albumin (up to 5.4 g/dL) did not interfere with the assay.

Interference studies with 31 different pharmaceuticals that might be found in the intended use population showed no interference at Total PSA concentrations of 0.3, 3.0 and 10 ng/mL.

Interference studies with purified Human anti-mouse antibody (HAMA) (up to 500 ng/mL) showed no interference at Total PSA concentrations of 0.3, 3.0 and 10 ng/mL. Due to the heterogeneous nature of HAMA, erroneous results may be obtained from patients that have undergone therapy or diagnostic procedures using monoclonal mouse antibody products.

Linearity

The linearity study was conducted using two K_2EDTA venous whole blood sample pools, containing 15.6 and 7.9 ng/mL, and were serially diluted with a K_2EDTA a venous whole blood sample containing zero total PSA in accordance with CLSI EP6-A. Weighted linear regression analysis was performed. The deviations from linearity for the measuring interval 0.08 – 15 ng/mL were <±15% for total PSA more than 0.25 ng/mL and <±0.04 ng/mL for total PSA less than 0.25 ng/mL.

High Dose Hook Effect

High dose hook effect for the Sangia Total PSA Test was evaluated by spiking K_2EDTA whole blood with PSA-ACT stock. No hook effect was detected up to at least 1250 ng/mL.

Equimolarity

Three samples containing total PSA with varying ratios of free-PSA to PSA-ACT were assayed and yielded the following results:

% of PSA-ACT	% of free PSA	0.25 ng/mL PSA		3.1 ng/mL PSA		12.8 ng/mL PSA	
		Mean (ng/mL)	Recovery (%)	Mean (ng/mL)	Recovery (%)	Mean (ng/mL)	Recovery (%)
0	100	0.27	107%	3.3	106%	12.3	96%
20	80	0.24	97%	3.1	101%	11.0	86%
50	50	0.23	91%	2.9	93%	11.8	91%
80	20	0.21	85%	3.1	101%	12.2	95%
100	0	0.25	100%	3.1	100%	12.8	100%

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Patent information related to Claros and Sangia are available at http://www.opko.com/products/patents/

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