A lateral flow, one step immunoassay for the rapid qualitative determination of PSA level in serum and/or whole blood

INTENDED USE

The RAP-2755 PSA Screen Rapid Test (Prostate Specific Antigen) is a rapid chromatographic immunoassay for the qualitative detection of Prostate Specific Antigen (PSA) in serum and/or whole blood. ***For in vitro diagnostic use***, except in the United States, where this kit is intended for professional and Research Use Only.

SUMMARY

Prostate specific antigen (PSA) is produced by prostate glandular and endothelial cells. PSA is a serum protease that is found only in the prostate within the epithelial cells of the acini and ducts. It is a single chain glycoprotein with a molecular weight of approximately 34 kDa. PSA exists in three major forms circulating in the serum. These forms are free PSA, PSA bound to α1 – Antichymotrypsin (PSA-ACT) and PSA complexed with α2–macroglobulin (PSA-MG). Normal PSA concentration in serum of healthy men is between 0.1-2.6 ng/ml. It can be elevated in malignant conditions such as prostate cancer, and in benign conditions such as benign prostatic hyperplasia and prostatitis. A PSA level of 4 to 10 ng/mL is considered to be in the "gray-zone" and above 10 ng/mL is highly indicative of cancer. A patient with a PSA value between 4-10 ng/mL this usually means further analysis of the prostate by biopsy. The PSA antigen test is the most valuable tool available for the diagnosis of early prostate cancer. Many studies have confirmed that the presence of PSA is the most useful and meaningful tumor marker known for prostate cancer and Benign Prostatic Hyperplasia (BPH).
The RAP-2755 PSA Screen Rapid Test is an immuno-chromatography rapid test. It is designed to detect human PSA in serum and/or whole blood with concentrations as low as 4 ng/ml within 10 minutes.

TEST PRINCIPLE

The RAP-2755 PSA Screen Rapid Test utilizes two-site sandwich immunoassay technology and specific antibodies to PSA for the qualitative detection of PSA in serum and/or whole blood. PSA specific antibodies are pre-coated onto membrane as a capture reagent in the test band region. During the assay the specimen reacts with anti-PSA gold-conjugate. The mixture then moves laterally on the membrane to the test region. The test region contains immobilized anti-PSA. If PSA is present in the specimen, a color band will form in the test region. The color band in the control region serves as a procedural control; a colored band will always appear in the control region indicating that proper volume of specimen has been added and that membrane wicking has occurred regardless the presence of PSA in sample.

REAGENTS AND MATERIALS PROVIDED

1. The RAP-2755 PSA Screen Rapid Test device contains PSA antibody coated on the membrane and PSA antibody conjugated with colloidal gold.
2. The test device is packed in a sealed, protective pouch.
3. Test Running Buffer, ready for use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Lancets (for fingerstick whole blood only)
2. Disposable heparinized capillary tubes and dispensing bulb (for fingerstick whole blood only).

STORAGE AND STABILITY

The RAP-2755 PSA Screen Rapid Test device is to be stored refrigerated or at room temperature (2-25°C) under dry conditions for the duration of its shelf life. The test device is stable through the expiration date printed on the sealed pouch. The test device must remain sealed in the pouch until use. **DO NOT FREEZE.**

PRECAUTION

1. For *in vitro* diagnostic use. In the United States, this kit is intended for Research Use Only.
2. Do not use after expiration date.
3. Do not eat, drink or smoke in the area where the specimens or kits are handled.
4. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.
5. Wear protective clothing such as laboratory coats, disposable gloves or eye protection when specimens are being tested.
6. High humidity and temperature can adversely affect results.

SPECIMEN COLLECTION AND STORAGE

The RAP-2755 PSA Screen Rapid Test is designed for the use of both whole blood and serum as the specimen. Collect blood and coagulate blood specimen following standard clinical procedure for proper collection of blood product specimens. If specimens are to be shipped, they should be packed in compliance with federal regulations covering the transportation of etiologic agents.

Whole blood specimen collection:

1. Collect fresh blood specimen just prior to using the assay. Specimens must be fresh.
2. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
3. 50 ul of blood is enough for assay.

Serum specimen collection:

1. Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear, non-hemolyzed specimens.
2. Testing should be performed immediately after specimen collection. Remove serum by centrifugation. Do not leave the specimens at room temperature for prolonged periods. Specimens can be stored refrigerated at 2-8°C up to 3 days. Freeze specimen at -20°C or lower for long term storage.
3. Avoid repeated freezing and thawing of specimens.

4. Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing.

ASSAY PROCEDURE

1. Do not open the foil pouch until ready to begin testing. Refrigerated test devices should be allowed to come to room temperature (15°- 28°C) before opening the pouch.
2. Remove the device from the protective pouch and label the device with specimen identification.
3. Add 50 ul of fresh blood or 25 ul of serum to the Sample Well (for Card) or Sample Pad (for Dipstick). Then add 3 drops (150 ul) of test running buffer into the sample well or sample pad.
4. Read the result within 10 minutes. Observe the colored band developed over the control region indicating the assay is complete.

***Note**: In order to prevent an incorrect reading, do not read the test results after 15 minutes. If the test is read after 15 minutes, the intensity of the colored lines may change or a new line may appear. To avoid confusion, discard all test devices after interpreting results.*

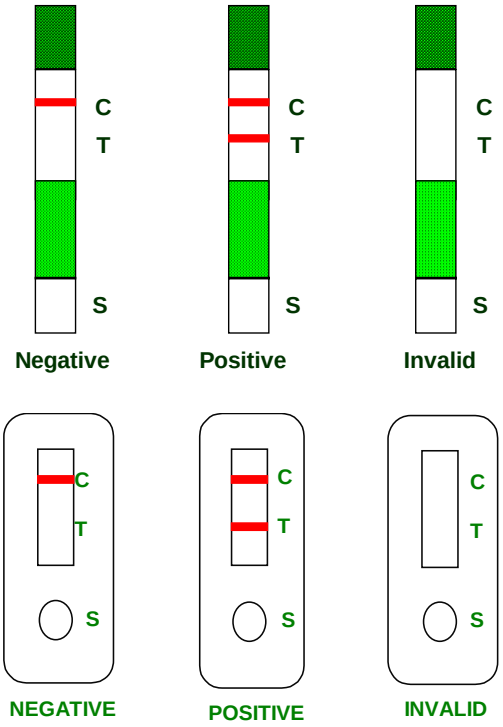
INTERPRETATION OF RESULT

Positive: Presence of two visible, pink-colored bands, one in the control region (C) and another in test region (T), indicates presence of PSA at concentrations of 4 ng/ml or higher in sample.

Negative: Presence of a single colored band in the control region (C) indicates the absence of PSA or that the concentration of PSA in sample is below the detection cut-off level (4 ng/ml).

Invalid: Results are invalid If after 10 minutes no band appears in the control region, or a band appears in the test region only. An invalid result may be due to improper assay procedures or damage to the device. The assay is inconclusive and the specimen should be repeated using a new test device.

Note: Do not interpret results after 15 minutes. The test band intensity may be weaker or stronger than that of the control band, but a very faint band in the test region indicates that the concentration of PSA in the sample is near the detection cut-off level (4 ng/ml). The sample should be re-tested or confirmed with a more specific method before a positive determination is made.



AFTER TESTING

The specimens may be potentially infectious. Avoid contact with skin by wearing gloves and proper laboratory attire. Properly handle and dispose of all test devices in an approved biohazard container. Residual sample should be disposed of in a medically approved manner after completion of all testing.

QUALITY CONTROL

The RAP-2755 PSA Screen Rapid Test devices have a built-in process Control Region (C). A pink band should always appear in this region regardless of the presence of PSA in sample. This pink process Control band verifies: 1) that sufficient serum volume was added, 2) that proper flow is obtained, and 3) that the reagents worked properly. If this band is missing, the test was not performed correctly or failed to function correctly. Such a test is inconclusive and the specimen should be repeated using a new test device.

Good laboratory practice requires use of control materials to ensure proper test device performance and reliability. The RAP-2755 PSA Screen Rapid Test has been standardized to Stanford's PSA Standard (LF500/L9010). Quality control standards are available for the validation of device functionality from commercial sources such as Sigma. Negative and positive controls containing PSA at various concentrations are available commercially. When testing the quality control standards, use the same assay procedure as with a serum or whole blood sample. It is recommended that control specimens be used for each new lot of kits.

PERFORMANCE CHARCATERISTICS

Sensitivity

The RAP-2755 PSA Screen Rapid Test has been designed for the detection of PSA in serum and/or whole blood at the detection sensitivity of 4ng/ml as indicated by the appearance of a colored band in the test region of the test device. Additionally, samples containing less than 4ng/ml may also produce a faint positive result. The following sensitivity studies were performed. A PSA standard panel was prepared by spiking pooled, fresh, normal human serum specimens with PSA standard (calibrated according to Stanford PSA Standard) to concentrations of 0, 0.5, 1, 2, 4, 8, 16 and 32ng/ml. In all, 50 test strip and 50 test card devices per standard level were tested in this procedure.

Table 1: The result of the sensitivity study for the test device

RAP-2755 PSA Test	PSA Concentration (ng/ml)							
	0	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**
Test No.	50	50	50	50	50	50	50	50
Positive	0	0	0	9	50	50	50	50
Negative	50	50	50	41	0	0	0	0

Summary of Sensitivity Results:

Concentrations of PSA equal to or lower than 1ng/ml were identified as negative results for all samples. Concentrations of PSA equal to or higher than 4ng/ml were identified as positive results for all samples. Therefore, the sensitivity of the RAP-2755 PSA Screen Rapid Test was determined to be 4ng/ml. Occasionally, specimens containing less than 4ng/ml may also yield positive results. Specimens containing high levels of PSA consistently gave positive results.

Specificity

Homologous Hormone Testing

The specificities of the RAP-2755 PSA Screen Rapid Test devices were tested for compounds related to or not associated with PSA as prepared in the normal human serum. The following compounds produced positive results at concentrations equal to or greater than the concentrations listed below:

α1–Antichymotrypsin PSA (PSA-ACT)	4ng/ml
Free PSA (F-PSA)	4ng/ml

The following compounds were found to have no impact on negative results when tested at levels up to the concentrations listed below:

Chrorionic Gonadotropin (hCG)	1,000mIU/ml
Prolactin (hPRL)	1,000mIU/ml
Prostatic Acid Phosphatase (PAP)	1,000ng/ml
α-Fetoprotein (hAFP)	1,000ng/ml
Carcinoembryonic Antigen (CEA)	1,000ng/ml

INTERFERENCE TESTING
The following substances were added to serum which had PSA levels of 0 and 10 ng/ml. None of the substances listed interfered with the RAP-2755 PSA Screen Rapid Test at concentrations tested.

Acetaminophen	20 mg/dl
Acetylsalicyclic Acid	20 mg/dl
Ampicillin	20 mg/dl
Ascorbic Acid	20 mg/dl
Atropine	20 mg/dl
Caffeine	20 mg/dl
Gentesic Acid	20 mg/dl
Glucose	2,000 mg/dl
Hemoglobin	1 mg/dl
Human Serum Protein	2,000 mg/dl
Tetracycline	20 mg/dl
Uric Acid	10 mg/dl

Precision
WITHIN LOT & INTER LOT REPRODUCIBILITY

Summary of Within-Lot Reproducibly
Three batches of normal human serum demonstrated to be negative for PSA were spiked with PSA to levels of 0, 1, 5 and 10 ng/ml. Single lots of the This PSA Screen Test devices were used to test for reproducibility of results. Five devices in each of three batches (for a total of fifteen devices from one lot) were tested per concentration. Results of within-lot reproducibility analytical studies clearly showed excellent repeatability for all 3 batches of positive and negative serum samples, using one lot of the This PSA Screen Rapid Test devices. The negative and positive values were correctly identified in 100% of cases.

Summary of Inter-Lot Reproducibility
To test inter-lot reproducibility, normal human serum known to be negative for PSA was spiked with PSA to levels of 0, 1, 5 and 10ng/ml. The samples were blinded by dispensing the mixed solutions into letter coded labeled vials, and were used to test three (3) lots of Test devices. Twenty (20) devices per concentration for each lot were tested. The results of these tests clearly demonstrate that there is no appreciable inter-lot variation when testing both positive and negative spiked samples across three (3) different lots of the RAP-2755 PSA Screen Rapid Test devices. The negative and positive values were correct in 100% of cases.

KIT COMPARISONS

Assay Comparisons & Equivalency

Accuracy and equivalency comparisons of both the RAP-2755 PSA Screen Rapid Test Card and Test Strip were evaluated against 162 individual in-house laboratory samples, as well as against 319 individual external EIA-certified clinical laboratory samples. The results have been tabulated below.

Table 2. PSA Test strip vs. Abbott EIA

RAP-2755 PSA Test strip	Abbott EIA (+)	Abbott EIA (-)	Row Totals
(+)	271	12	283
(-)	4	194	198
Col. Totals	275	206	481

When compared to EIA Assay, the percent agreement with This PSA Screen Test Strip positive samples was 271/275 or 98.6%. Negative samples recovered at 194/206 or 94.2%, while the overall relative accuracy obtained was 465/481 or 96.7%.

Table 3. PSA Card vs. Abbott EIA

RAP-2755 PSA Test	Abbott EIA (+)	Abbott EIA (-)	Row Totals
(+)	273	9	282
(-)	2	197	199
Col. Totals	275	206	481

When compared to EIA Assay, the percent agreement with the RAP-2755 PSA Screen Rapid Test Card positive samples was 273/275 or 99.3%. Negative samples recovered at 197/206 or 95.6%, while the overall relative accuracy obtained was 470/481 or 97.7%.

LIMITATIONS

1. The test result will only indicate the qualitative level of PSA in the specimen and should not be used as the sole criteria for the diagnosis of Prostate Cancer.
2. A significant numbers of patients with BPH (more that 15%) and less than 1% of healthy individuals have elevated PSA. Even if the test results are positive, further clinical evaluation should be considered with other clinical information available to the physician.
3. PSA levels may be unreliable in patients who receive hormone therapy or prostate gland manipulation.

EXPECTED VALUES

The cut off value of the RAP-2755 PSA Screen Rapid Test is generally agreed to be 4 ng/mL. The warning value is 10 ng/mL. The test has been compared with a leading commercial PSA EIA test. The correlation between these two systems is over 96.7% (for dipstick) or 97.7% (for the card device).

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Ver. 0403/101006
Rev. 4/4/08~sd