Laboratory Procedure Manual

Analyte: **Complex Prostate-Specific Antigen (PSA)**

Matrix: **Serum**

Method: **Advia Centaur**

Method No.:

Revised:

as performed by: *University of Washington Medical Center*
Department of Laboratory Medicine
Immunology Division

Contact: *Kathleen Hutchinson M.S., M.T. (ASCP) or*
Mark Wener, M.D., Director

Important Information for Users

The University of Washington Medical Center Laboratory periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

File Name	Variable Name	SAS Label
PSA_E	LBXPS4	PSA, complex (ng/mL)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Prostate specific antigen (PSA) is a serine protease produced by epithelial cells of the prostate gland. The proteolytic activity of PSA is inhibited in the bloodstream by the formation of complexes with serine protease inhibitors. The major immunoreactive forms of serum PSA include free PSA and complexes of PSA, primarily with α-1-antichymotrypsin (ACT), and small amounts of α-1-antitrypsin and inter-α-trypsin inhibitor. PSA also forms complexes with α-2-macroglobulin which is not immunoreactive due to the encapsulation of PSA by the α-2-macroglobulin.

The ADVIA Centaur cPSA assay quantitatively measures complexed prostate-specific antigen (cPSA) in human serum. Free PSA present in the sample is prevented from reacting with the total PSA antibodies by incubating the sample at 37°C with a free-PSA-specific monoclonal mouse antibody (Pretreatment Reagent), which blocks the free PSA so that it is nonreactive in the ADVIA Centaur cPSA assay. The cPSA in the sample is then measured in the two-site sandwich immunoassay using direct chemiluminometric technology, which uses constant amounts of two antibodies. The first antibody, in the Lite Reagent, is a polyclonal goat anti-PSA antibody labeled with acridinium ester. The second antibody, in the Solid Phase, is a monoclonal mouse anti-PSA antibody, which is covalently coupled to paramagnetic particles. The immunocomplexes of sample cPSA sandwiched and coupled to paramagnetic particles are magnetically separated from unbound components and washed in the cuvette incubation ring. The addition of hydroxyl groups to complete the flash reaction is accomplished by the addition of Acid and Base. The chemiluminescent reaction occurs in the luminometer. The photomultiplier tube (PMT) measures the chemical light reaction that takes place. A direct relationship exists between the amount of cPSA present in the patient sample and the amount of relative light units (RLUs) detected by the system. The amount of cPSA in the sample is determined by means of a stored, multi-point calibration curve.

This assay was developed to aid in the detection of prostrate cancer in men. Biopsy of the prostate is required for the diagnosis of prostate cancer. Further studies are needed to confirm that measuring cPSA is more sensitive than total PSA levels in cancer detection. The concentration of cPSA in a given specimen, as determined by assays from different manufacturers, can vary due to differences in assay methods and reagent specificity and cannot be used interchangeably.

2. SAFETY PRECAUTIONS

Consider all samples received for analysis potentially positive for infectious agents including HIV and the hepatitis B virus. Observe universal precautions. Wear gloves, lab coat, and safety glasses when handling all human blood products and infectious viruses. Place disposable plastic, glass, paper, and gloves that contact blood in a biohazard bag or discard pan to be autoclaved. Disinfect all work surfaces with staphene solution. Dispose of all biological samples and diluted specimens in a biohazard bag at the end of the analysis.

Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wash hands thoroughly after removal or personal protective devices used in handling specimens and kit reagents.

Material safety data sheets for all reagents used in the performance of this assay,

including but limited to staphene, and sodium azide are kept in the Immunology Division, University of Washington Medical Center (UWMC).

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

a. Each shipment of specimens received from the NHANES mobile unit arrives with a corresponding transmittal sheet and an electronic version of the shipping/resulting file. The file structure is determined by NHANES and is described in the National Health and Nutrition Examination Survey (NHANES) Contract Laboratory Manual.

b. After the testing is completed results from the Advia Centaur are transferred to the laboratory server system, which is backed up daily. This instrument file contains the following information for each sample, control and calibrator tested.

Result ID
Result (Date) Time
Specimen ID
Test Name
Units
Dose
Index
Interpretation
RLU
Replicate Number
Replicate ID
Comment 1
Comment 2
Comment 3
Primary Reagent Lot
Ancillary Reagent Lot

c. QC results are transferred to an Excel file using laboratory-developed software. This file calculates the QC statistics, plots Levey-Jennings charts, displays relevant instrument flags, tracks reagent lots and recent calibrations. QC results are reviewed prior to resulting samples.

d. Sample results are transferred to an Excel file using laboratory-developed software that enters results after matching sample identifiers from the instrument file with those provided in the NHANES shipping/resulting file. This Excel file is formatted to match the NHANES shipping/resulting file and the program uses the conventions outlined in the NHANES Contract Laboratory Manual.

e. Data entry is checked for errors.

f. After the total and free PSA has also been completed, resulted, and checked, the result file is transmitted electronically to NHANES WESTAT. Electronic and hard copies of the files are kept in the laboratory.

g. Technical support for this system is provided by Westat, Rockville, MD (1-301-294-2036)

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

a. No special instructions such as fasting or special diets are required.

b. Serum is the required specimen type. Plasma is not acceptable. If testing is to be done within 48 hours, samples can be refrigerated at 2 to 8°C. Freeze at -20ºC or colder for longer storage.

c. Blood should be collected aseptically and the serum separated by standard laboratory techniques. Specimens may be collected by using regular or serum-separator Vacutainers. Serum should be separated from the cells within 2 hours of collection.

d. The requested sample volume for the assay is 1.0 mL, and the minimum sample volume is 0.5 mL.

e. Specimens may be stored in glass or plastic vials, as long as the vials are tightly sealed to prevent desiccation of the sample.

f. Turbid samples or those with particulate matter should be centrifuged prior to assay.

g. More than one freeze-thaw cycles is not recommended.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6. PREPARATION OF REAGENTS, CALIBRATORS (STANDARDS), CONTROLS, AND ALL OTHER MATERIALS; EQUIPMENT AND INSTRUMENTATION

a. Instrumentation

1. Advia Centaur Immunoassay System (Siemens Healthcare Diagnostics, 1717 Deerfield Road, Deerfield, IL 60015-0778, USA)

   The Advia Centaur is a fully automated, random access, instrument that features on-board storage of reagent packs in a refrigerated compartment; sample and reagent delivery, mixing, barcode identification of specimens and reagent packs; temperature controlled reaction reactions; and measurement and analysis of the light signal (RLU) generated by the chemiluminescent reaction.

   The complex PSA assay parameter settings for the instrument are as follows:

Parameter	Setting
Sample Volume Requirements	
Minimum sample volume	145 ul
Sample volume used for testing	35 ul
No. of Standard Points	
Stored curve established by manufacturer	6
Calibration performed by lab	2

Standard Curve Measuring Range (At initial dilution; approximate values, range is dependent upon standard value)	0.06 – 80 ng/mL

2. Computers (Dell Computer Corporation, Round Rock, Texas).

3. Centrifuge (Jouan Inc., Winchester, VA)

b. Equipment

1. Centaur cuvettes, tips, and sample cups (Siemens Healthcare Diagnostics, Deerfield, IL)

2. Latex gloves, disposable (Any manufacturer).

3. Pipettes and tips (Rainin, Emeryville, CA)

c. Reagents

All reagents are purchased from. Siemens Healthcare Diagnostics, Deerfield, IL

1. cPSA Reagent Pack (Primary Reagent): Cat. No. 124830: 100 determinations.
   Lite Reagent: Polyclonal goat anti-PSA antibody (~77 ng/mL) labeled with acridinium ester in buffered saline with bovine serum albumin and preservatives
   Solid Phase: Monoclonal mouse anti-PSA antibody (~25 μg/mL) covalently coupled to paramagnetic particles in buffered saline with bovine serum albumin and preservatives

   Provided ready to use. Store upright and refrigerate at 2 to 10°C. Stable until the expiration date stated on the label when stored at 2 to 10°C. Stable at 2 to 10°C for 28 days after initial use. Shake pack well for 15 seconds then tap 5 times when loading instrument. Inspect for suspension of PMPs.

2. cPSA Pre-treatment Reagent (Ancillary Reagent): Cat. No. 125308: 500 tests
   Free-PSA-specific monoclonal mouse anti-PSA antibody (~50 μg/mL) in buffered saline with bovine serum albumin and preservatives

   Provided ready to use. Store upright and refrigerate at 2 to 10°C. Stable until the expiration date stated on the label when stored at 2 to 10°C. Stable 41 days after initial use.

3. Multi-Dil 2 (Ancillary Reagent): Cat No. 110314, 10mL
   Goat serum with sodium azide (0.1%) and preservatives

   Provided ready to use. Store upright and refrigerate at 2 to 10°C. Stable until the expiration date stated on the label when stored at 2 to 10°C. Stable at 2 to 10°C for 28 days after initial use.

4. Acid Reagent: Cat # 112219 1500mL
   0.5% H_20_2, 0.1N HNO_3

Ready to use. Change base at same time. Store at room temperature. Stable 30 days after opening.

5. Base Reagent: Cat # 112219 1500mL
   0.25N NaOH and surfactant

   Ready to use. Change acid at same time. Store at room temperature. Stable 30 days after opening.

6. Cleaning Solution Concentrate: Cat # 112748 12 vials 70mL ea.
   Sodium hypochlorite (0.82mol/L)

   Used to make working cleaning solution. Store at 2 to 10°C. Stable until expiration date on vial.

7. Working Cleaning Solution
   1 vial of cleaning solution concentrate diluted to 2.0 liters with dH2O.

   Store at room temperature on the instrument. Stable 7 days after preparation.

d. Standards/Calibration Preparation

1. cPSA Calibrators: Cat No. 125014, 2mL
   Low: Approximately 0.00 ng/mL
   High Approximately 50 ng/mL
   Concentrations are lot dependent and established by Siemens. Used for assay calibration.

   Unopened vials: store 2 to 10°C. Reconstitute with 2.0 mL dH2O. Let stand 20 min. Stable 21 days after reconstitution. Store 2 to 10°C.

2. cPSA MCM: Cat # 124828.
   Master Curve Materials. Approximate values:100, 60, 40, 20, 10, 5, 1.0, 0.5, 0.0 ng/mL. Concentrations are lot dependent and established by Siemens. Used for AMR validation.

   Unopened vials: store 2 to 10°C. Reconstitute with 1.0 mL dH2O. Let stand 20min. Stable 7 days after reconstitution.

e. Preparation of Quality Control Materials

Two different levels of serum controls are run with each run. These controls may be purchased or prepared in-house. Commercial controls are stored and used according to the manufacturer's recommendations. In house controls are stored frozen (-20ºC or colder). Frozen aliquots should be thawed and mixed well. Once thawed, the controls are stored at 2-8 ºC. All controls are used within their stated expiration dates.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a. Calibration

Example cPSA Calibration:

Advia Centaur *TM* Automated Chemiluminescence System Centaur 3269

24 Jan 08 08:46

Calibration Data

	Reagent Lot	Calibrator	Calibrator Lot	Calibrated
cPSA	202	CALY	CY 38	24 Jan 08 08:39

Calibration Data

Calibrated	Status	Flag	Cal Due	Calibrator	Expiration
24 Jan 08 08:39	Valid		21 Feb 08 08:39	CALY	27 Mar 08
Comments			Operator	Date	

Low Cal

	Value		Date	Comments
Conc	0.00	ng/mL		
Replicate 1	2590		24 Jan 08 08:39	
Replicate 2	2702		24 Jan 08 08:39	
Mean	2646.00		24 Jan 08 08:39	
CV	3.0			
Acceptable CV	12.3			

High Cal

	Value		Date	Comments
Conc	55.90	ng/mL		
Replicate 1	1087392		24 Jan 08 08:39	
Replicate 2	1033930		24 Jan 08 08:40	
Mean	1060661.00		24 Jan 08 08:40	
CV	3.6			
Acceptable CV	7.5			

Master Curve Data

Master Curve Units	Concentration	RLU	Reagent Lot	Expiration
ng/mL	0.00	3518	202	11 Sep 08
	0.61	21910		
	4.34	42099		
	4.13	110140		
	8.46	216938		
	20.90	503954		
	41.90	856082		
	64.30	1132358		
	109.00	1494098		

Complex PSA concentrations are calculated by using a calibration curve. This curve has a direct relationship of measured light produced (RLU) to concentration of free cPSA in the serum sample. Serum results are expressed as ng/mL.

Calibrators are traceable by to the manufacturer's working calibrators. The assigned values were established using an Internal standard manufactured by Siemens using highly purified PSA-ACT (PSA complexed with α-1-antichymotrypsin). Values assigned by other methodologies may be different.

An active calibration curve is required for all tests. For the cPSA, calibration is required every 28 days or whenever new lot numbers of reagents are placed into use. Refer to the Operator's Guide and Reference Manual for complete instructions on calibration procedures.

b. Verification

1. Two levels of control are run for each test series. If, within a testing series, these controls do not conform to specifications as defined in the quality control manual, the entire series is invalidated.

2. New lot numbers of calibrator are verified by running 100 or more samples tested on the previous lot number. The correlation is analyzed using one or more linear regression formulas.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. Preliminaries

1. Bring all controls and patient specimens to room temperature before use. Mix any specimens or controls that have been frozen. Centrifuge samples with particulate matter prior to testing.

2. Perform the Maintenance procedures as indicated by the Centaur. Daily maintenance is performed by the system automatically.

3. Review the Reagent status for pack stabilities, number of tests and lots available and calibration due dates. Calibrate if indicated.

b. Instrument Operation (see operator's manual for details).

1. Check sample volume to make sure that there is sufficient volume to perform testing. Gently mix, uncap and load specimens into specimen racks, with the barcode in the open slot. Make sure there are no bubbles. If the barcode is not reading properly, sample IDs can be entered manually. Load the racks onto the instrument.

2. Select the cPSA test. Testing is done in singlicate. Select the sample(s) to be used for the random repeat testing.

3. The instrument will automatically dilute samples above the analyte measuring range (AMR) using a 1:2 dilution. If needed, the operator can program the instrument to perform a 1:5 dilution. If cPSA result is still too high, an off-line dilution using cPSA diluent should be made and the dilution factor entered into the instrument. The instrument automatically calculates all results. After testing is completed, results are printed and review by the technologist.

4. Remove specimens and controls soon after the instrument finishes pipetting from the sample. Return controls to the refrigerator and refreeze specimens.

5. Perform scheduled instrument maintenance (daily, weekly, and monthly) as outlined on the maintenance log. See the operator's manual for specific instructions.

c. Recording of Data

1. Using a lab developed program, specimen results are transferred from the instrument data file into the assay specific results table created from the send file corresponding to the specific sample box. The file format is Excel (Microsoft Corporation, Redmond WA). A copy of this file is printed out and checked for accuracy of data entry.

2. Control results are entered to the Assay Specific QC/Levy-Jennings Table using the Excel program. Compliance with the Westgard rules is evaluated. A copy of this table is printed out and checked for accuracy of data entry.

d. Replacement and Periodic Maintenance of Key Components

1. Daily Maintenance:
   Start-up performed by the technologist:
   -Check system reagents and supplies and replace as needed.
   -Check the expiration date of the current calibration.
   -Fill the water supply with distilled water.
   -Empty the waste container.
   -Prime the system.
   Shut-down performed by the technologist:
   -Resupply system reagents and supplies as needed.
   -Check waste containers, empty if needed
   Daily Cleaning performed by the instrument:
   -The Centaur is programmed to perform an automatic cleaning procedure following each day's run which includes cleaning the probes, the rinse and washes stations and associated tubing.
2. Weekly Maintenance:
   -Replace the cleaning solution every four days
   -Empty the water trap.
   -Replace and clean the water bottle and reservoir. Clean the sensors.
   -Prime the water reservoir.
3. Monthly Maintenance]
   -Clean the exterior of the aspirate, reagent and ancillary probes.
   -Clean the air filter.
   -Perform the Monthly Cleaning Procedure which pumps cleaning solution throughout the system.
   -Check and clean probe rinse stations, probe shutters, covers, pointing device, cuvette waste area, reagent compartment, and sample racks as needed.

-Replace the acid and base reagents.
-Perform database management.

4. Periodic Maintenance to be performed by the manufacturer's service engineer.

e. Calculations

Patient test results are determined automatically by the system software. The amount of analyte in a sample is determined from the measured light production by means of a stored nonlinear calibration curve. Patient test results can be reviewed using the Sample Results screen. Refer to the Operator's Guide for complete instructions on reviewing results.

9. REPORTABLE RANGE OF TEST RESULTS

Results are reported to the nearest hundredth (0.01). The lowest reportable cPSA result is 0.60 ng/mL. Results above 80.00 ng/mL) are repeated with dilution until a valid number is obtained. Estimates of imprecision can be generated from long-term quality control pool results.

10. QUALITY CONTROL (QC) PROCEDURES

a. Bench quality controls are used in this analytical method. Bench quality control specimens are tested with each analytical run (a set of consecutive assays performed without interruption) so that judgments may be made on the day of analysis. The data from these materials are then used in estimating methodological imprecision and in assessing the magnitude of any time-associated trends.

b. The bench controls are purchased in sufficient quantity to provide serum samples for all the assays for approximately 1 year. Ranges are established after 20 parallel runs with previously established controls. The quality control pools comprise two levels of concentration spanning the low and high ranges for cPSA.

c. Bench quality controls are placed at the beginning of each analytical run. After analysis, the long-term quality control charts (Levey-Jennings) for each control material are consulted to determine if the system is "in control." The Levey Jennings chart plots the quality control material observations on the y-axis and the date of the observation on the x-axis. Quality control material observations are compared with the 95% and 99% confidence limits as well as with the center line (the overall mean of the characterization runs) prior to reporting any results. The system is out of control if any of the following events occur for any one of the quality control materials:

-The observation from a single pool falls outside the 99% confidence limits.
-The observations from two pools fall either both above or both below the 95% confidence limits.
-The observations from eight successive runs for one pool fall either all above or all below the center-line and the current result is above or below the 95% confidence limits.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

If the run is declared "out of control", the system (instrument, calibration standards, etc.) is investigated to determine the root of the problem before any results are released. Consult with the supervisor for appropriate actions.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

a. The lowest reportable value is approximately 0.6 ng/mL. According to the manufacturer, 0.03 ng/mL is the lowest detectable level of cPSA distinguishable from zero with 95% confidence.

b. There is no upper limit of the reportable values. Samples with results under 8000 ng/mL do not demonstrate high dose hook errors.

c. The cPSA results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information.

d. Patients treated with anti-androgens and LHRH agonists and antagonists may exhibit markedly reduced levels of cPSA. Care should be taken when interpreting values from these individuals.

e. According to the manufacturer the following substances showed less than 5% change in results:
-Hemoglobin up to 500 mg/dL
-Bilirubin up to 40 mg/dL
-Triglycerides up to 1000 mg/dL
-Protein up to 12.5 g/dL

f. In-house interference studies showed:
-Immune complexes: No effect observed. 3 specimens with 1 -20 cPSA levels were spiked a sample with high levels of immune complexes, all showed >94% of expected result.
-Rheumatoid factor: No effect observed. 3 specimens with 1 -20 cPSA levels were spiked with each of two samples with very high rheumatoid factor levels; all showed >93% of expected result.
-Polyclonal hypergammaglobulinemia: Potential interference with high levels. 3 specimens with 1 -20 cPSA levels were spiked with each of two samples with gammaglobulin levels >2.5g/dL; 2 specimens showed 78 and 81% of expected. The remainder showed >94% of expected result.

g. There are no known cross-reactants for cPSA.

h. For assay employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients that have been regularly exposed to animals or have received immunotherapy or diagnostics procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interferes with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may also be present in patient samples. Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

13. REFERENCE RANGES (NORMAL VALUES)

0 – 3.59 ng/mL

Based on manufacturer's studies of the results measured in the serum of 199 apparently healthy male subjects ranging in age from 42 to 92 years old.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable to this procedure.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens should be maintained at 20-25 ^{o}C during testing. After testing, the samples are stored at -70 ^{o}C or colder.

16. ALTERNATIVE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

There are no acceptable alternative methods of analysis. Specimens may be stored at 4-8 ^{o}C for no longer than 2 days. Otherwise, specimens should be stored -70 ^{o}C or colder until the system is returned to functionality.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable to this procedure.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

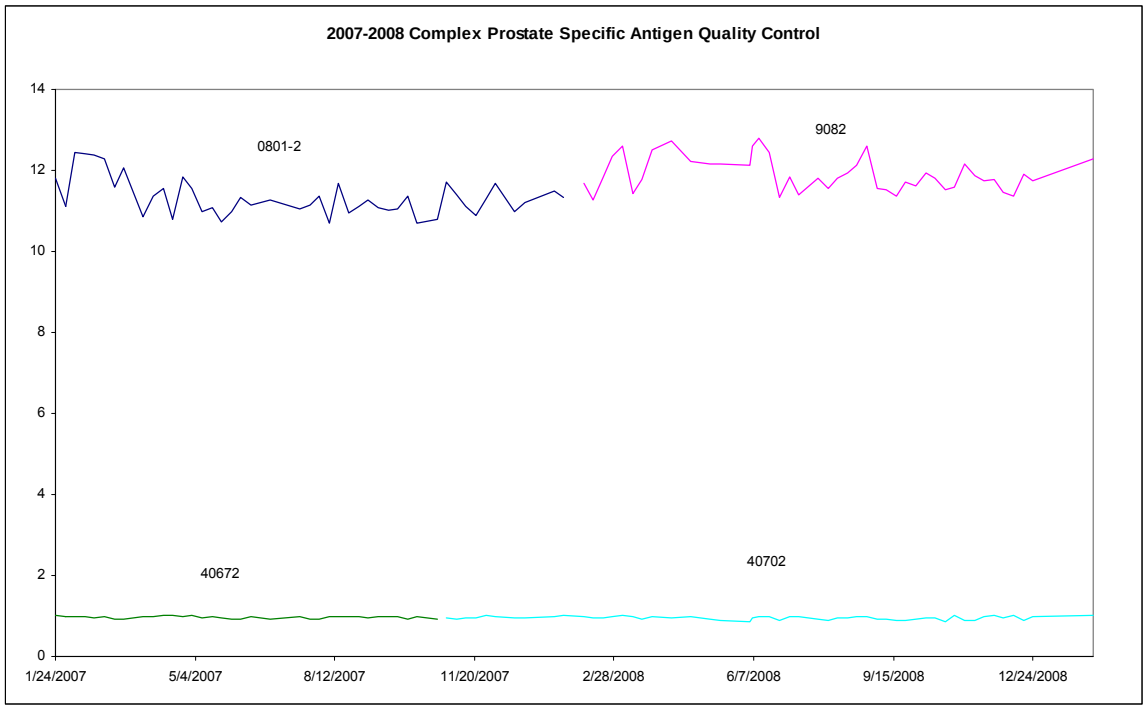
Standard record keeping should be used for tracking specimens. Samples are inspected upon arrival and new boxes are added to an Excel worksheet (sample log) used to track boxes. This sample log is used to track the status of testing and resulting.

The residual serum is stored at $\leq$ -70 ^{o} C for 6 months after analysis, then it is returned to the NHANES Repository in Rockville, MD for long-term storage.

19. SUMMARY STATISTICS AND QC GRAPHS

Summary Statistics for Complex Prostate Specific Antigen by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
40672	35	1/24/2007	10/24/2007	0.967	0.034	3.5
0801-2	45	1/24/2007	1/23/2008	11.329	0.458	4.0
40702	53	10/31/2007	2/5/2009	0.950	0.043	4.5
9082	43	2/6/2008	2/5/2009	11.910	0.417	3.5



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1. Manufacturer Information:
   -Advia Centaur Immunoassay System Operator's Guide and Reference Manual
   -cPSA kit inserts, Siemens Healthcare Diagnostics, Deerfield, IL

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