

Hybritech free Prostate Specific Antigen (free PSA) on the Beckman Access

University of Washington Medical Center
Department of Laboratory Medicine
Immunology Division
Director: Mark Wener M.D.
Supervisor: Phyllis Daum M.T. (ASCP)
Author: Kathleen Hutchinson M.S., M.T. (ASCP)

0. Public Release Data Set Information

This document details the Lab Protocol for NHANES 2001-2002 data.

A list of the released analytes follows:

Lab	Analyte	SAS Label	Description
l11psa_b	LBXP2	PSA, free (ng/mL)	Prostate Specific Antigen, free

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The Access Hybritech free PSA assay is a two-site immunoenzymatic "sandwich" assay. A sample is added to a reaction vessel with mouse monoclonal anti-free PSA alkaline phosphatase conjugate, and paramagnetic particles coated with a second mouse monoclonal anti-free PSA antibody. The free PSA in the sample binds to the immobilized monoclonal anti-free PSA on the solid phase while, at the same time, the monoclonal anti-PSA conjugate reacts with a different antigenic site on the sample free PSA. Separation in a magnetic field and washing removes material not bound to the solid phase. A chemiluminescent substrate, Lumi-Phos** 530 is added to the reaction vessel and light generated by the reaction is measured with a luminometer. The light production is proportional to the concentration of free PSA in the sample. The amount of analyte in the sample is determined by means of a stored, multi-point calibration curve. Total PSA values are obtained using the Hybritech PSA method on the Beckman Access (alternate methods are not acceptable).

Prostate cancer is one of the most common types of cancer found in men. Because the incidence of cancer increases with age, the number of newly diagnosed cases and death continues to rise as the life expectancy of the general population increases.

Prostate-specific antigen was identified and purified by Wang and co-workers in 1979. PSA is a single chain glycoprotein with a molecular weight of approximately 34,000 daltons, containing 7% carbohydrate by weight. Immunohistochemical studies have shown that PSA is found predominately in the cytoplasm of prostatic acinar cells and ductal epithelium. PSA is present in normal benign hyperplastic and malignant prostatic tissue, and also in prostatic fluid and seminal plasma. PSA has not been detected in cancers of the colon, rectum, stomach, pancreas or thyroid.

PSA exists primarily as three forms in serum. The major form of PSA is believed to be enveloped by alpha-2 macroglobulin. This form lacks immunoreactivity. The second major form of PSA in serum is complexed to another protease inhibitor, alpha-1 antichymotrypsin (ACT). ACT binds only to the active site of PSA, but PSA remains immunologically detectable. The third form of PSA is not complexed to a protease inhibitor and is termed free PSA. This form is also immunologically detectable.

Measurement of free PSA and PSA-ACT may be of clinical value in distinguishing prostate cancer from BPH, when used in conjunction with the total PSA result. Among patients with a total PSA in the 4.0 to 10.0 ng/ml range, recent studies indicate that men with prostate cancer tend to have a lower free PSA/total PSA ratio than those with BPH.

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 IV mobile unit arrives with a corresponding transmittal sheet and a Send File (a comma delineated text file) transmitted electronically (labeled *boxnum.shp*). This file contains the following information:

Send File

Field	Type
Sample ID	XXXXXXXXXX
Slot Number	XXX
Sample Collection Date	mm/dd/yyyy hh:mm:ss
MEC Comment Code	XX

- b. The information from the shipping file is imported into a result file with the following format:

Results File: PSA/free PSA-Vessel ID 75

Field	Format
Sample ID	XXXXXXXXXX
Slot Number	XXX
Sample Collection Date	mm/dd/yyyy hh:mm:ss
MEC Comment Code	XX
Date of Receipt	Mmddyyyy
PSA Total Date of Analysis	Mmddyyyy
PSA Total Analyst ID	XXX
PSA Total Run Number	{test code}mmddy.x(letter)
PSA Total (ng/mL)	XXXXX.X
PSA Total comment	XX
PSA Total 2.5% repeat (ng/mL)	XXXXX.X
PSA Free Date of Analysis	Mmddyyyy
PSA Free Analyst ID	XXX
PSA Free Run Number	{test code}mmddy.x(letter)
PSA Free (ng/mL)	XXXXX.XX
PSA Free comment	XX
PSA Free 2.5% repeat (ng/mL)	XXXXX.XX
PSA ratio (%)	XXX

- c. After the testing is completed, the run number, date of analysis, free PSA result, free PSA comment, free PSA analyst, and the free PSA 2.5% repeat results are entered into the result file.
- d. Data entry is checked for errors.
- e. After the total PSA testing 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.
- f. 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. Specimens for PSA testing should be drawn prior to prostatic manipulations such as digital rectal exams (DRE), prostatic massage, transrectal ultrasound, and protastic biopsy. DRE may cause a transient increase in serum PSA levels. Transrectal needle biopsy has been shown to cause persisting PSA elevations. A 6-week waiting period between needle biopsy and PSA sampling is recommended. The test is usually performed on samples with total PSA results between 4.0 – 10.0 ng/mL.
- b. Serum is the required specimen type; plasma should not be used. If testing is to be done within 24 hours, samples can be refrigerated at 2 to 8°C. Freeze at -20°C 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 60 minutes of collection.
- d. The requested sample volume for the assay is 1.0 mL, and the minimum sample volume is 0.3 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. Repeated freeze-thaw cycles do not affect free PSA, total PSA, or percent free PSA, but prompt refreezing of the thawed samples is 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. Beckman Access or Access II Immunoassay System (Beckman Coulter, Fullerton, CA.)

The Beckman Access is a fully automated, random access, instrument that features on-board storage of reagent packs in a refrigerated compartment; an ultrasonic probe tip for level sense detection, sample and reagent delivery, mixing, and probe cleaning to minimize carryover; barcode identification of specimens and reagent packs; temperature controlled reaction reactions; and measurement and analysis of the light signal generated by the chemiluminescent reaction (RLU) using a smoothing cubic spline math model.

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

Parameter	Setting
Sample Volume Requirements	
Minimum sample volume	175 ul
Sample volume used for testing	25 ul
No. of Standard Points	6
Calibration curve calculation	Smoothing cubic spline math model
Standard Curve Measuring Range (At initial dilution; approximate values, range is dependent upon standard value)	0.01 - 20 ng/mL

2. Hewlett Packard DeskJet printer (Hewlett Packard, Boise, ID)
3. Computers (Dell Computer Corporation, Round Rock, Texas).
4. Centrifuge (Jouan Inc., Winchester, VA)

b. Equipment

1. Reaction Vessels (Beckman Coulter, Fullerton, CA)
2. Sample Cups (Fisher Scientific, Pittsburgh, PA)
3. Latex gloves, disposable (Any manufacturer).
4. Pipettes and tips (Rainin, Emeryville, CA)

c. Reagents

All reagents are purchased from Beckman Coulter, Fullerton, CA.

1. R1: Access Hybritech free PSA reagent packs:
Cat. No. 37210: 100 determinations, 50 tests/pack

Provided ready to use. Store upright and refrigerate packs at 2 to 10°C. Packs must be refrigerated at 2 to 10°C for a minimum of two hours before use on the instrument. Stable until the expiration date stated on the label when stored at 2 to 10°C. After initial use, the pack is stable at 2 to 10°C for 28 days. Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.

R1a: Paramagnetic particles coated with burro anti-goat, goat anti-biotin, and biotinylated mouse monoclonal anti-PSA in Tris buffered saline with surfactant, bovine serum albumin (BSA), < 0.1% sodium azide, and 0.1% ProClin**300.

R1b: Mouse monoclonal anti-free PSA alkaline phosphatase (bovine) conjugate diluted in phosphate buffered saline, with surfactant, BSA, protein (mouse), < 0.1% sodium azide and 0.25% ProClin**300.

2. Access Substrate Cat. No. 81906: 4 x 130 ml

Lumi-Phos* 530(buffered solution containing dioxetane. Lumigen* PPD, fluorescer, and surfactant. Store at 2-8 °C. Stable until expiration date on label when unopened. Bring to room temperature (15 – 30 °C) at least 18 hours before use. Stable for 14 days at room temperature or after bottle has been opened.

3. Access Wash Buffer: Cat # 81907

Tris buffered saline, surfactant, <0.1% sodium azide, and 0.1% Pro Clin* 300. Provided ready to use. Store at room temperature (15 – 30 °C), stable until expiration date on label.

4. Hybritech PSA Sample Diluent: Cat # 37206

Buffered BSA, < 0.1% sodium azide, 0.5% ProClin**300. Provided ready to use. Allow the contents to stand for 10 minutes at room temperature and mix by gently inverting prior to use. Avoid bubble formation. Stable until the expiration date when stored at 2-8 °C.

d. Standards/Calibration Preparation

Access Hybritech free PSA Calibrators Cat. No. 37215. S0: 5.0 ml/vial, S1-S5: 2.5 ml/vial. Provided ready to use. Store at 2 to 10°C. Mix contents by gently inverting before use. Avoid bubble formation. Stable until the expiration date stated on the vial labels when stored at 2 to 10°C. Control values out of range are a sign of possible deterioration.

- S0: Buffered BSA, < 0.1% sodium azide, 0.5% ProClin**300. Contains 0.0 ng/ml of free PSA.
- S1, S2, S3, S4, S5: Human free PSA at levels of approximately 0.5, 2.0, 5.0, 10.0, and 20.0 ng/ml, respectively, in buffered BSA, CQ. < 0.1% sodium azide, 0.5% ProClin**300. Refer to calibration card for exact concentrations.
- Calibration Card (with actual calibrator concentration information)

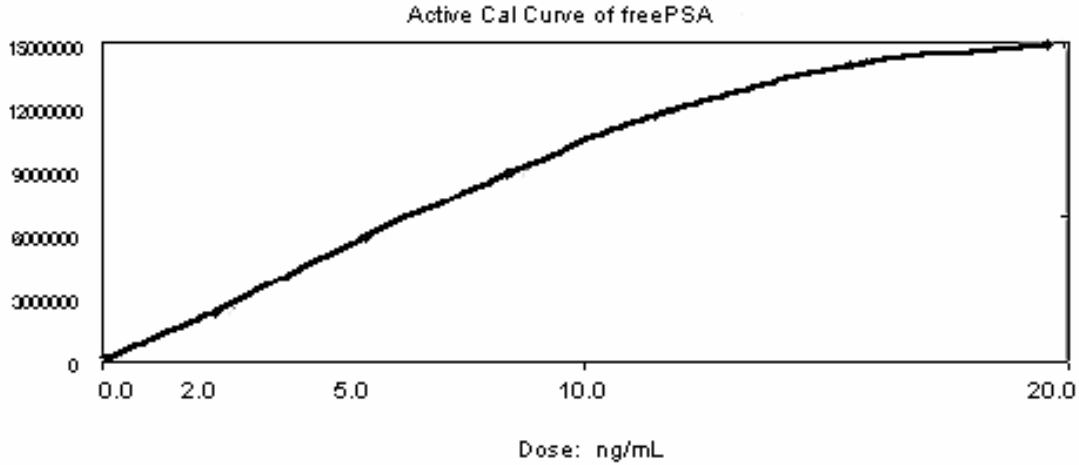
e. Preparation of Quality Control Materials

Two different levels of serum controls are run with each run. The controls are prepared in-house. The controls are stored frozen (-20°C or colder). Once thawed, the controls are stored at 2-8 °C. The controls are stable until the expiration date on the label if frozen. Once thawed, they are stable for 30 days.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a. Calibration Curve

Example free PSA Calibration:



Test: freePSA

Cal Lot: 006858

Reagent Lot: 006857

Status: Accepted

Calibrated: 01-19-2001 08:56am

Curve Exp.: 02-16-2001 08:56 am

Stated Conc. ng/mL	Mean				Calc. Conc. ng/mL	Mean Calc. Conc. ng/mL	Flags
	RLU	RLU	1SD	% CV			
0.00	16757				0.00		
0.00	16034				No Value		
		16395	512	3.12		0.00	
0.50	336982				0.49		
0.50	337631				0.49		
		337306	459	0.14		0.49	
2.00	1398560				2.10		
2.00	1323150				1.98		
		1360855	53323	3.92		2.04	
4.99	3311440				5.14		
4.99	3290410				5.10		
		3300925	14870	0.45		5.12	
10.20	6282140				10.26		
10.20	5971300				9.72		
		6126720	219797	3.59		9.99	
19.80	11556300				19.22		
19.80	12296000				No Value		
		11926150	523047	4.39		19.22	

Free PSA concentrations are calculated by using a calibration curve. This method utilizes a smoothing cubic spline math model with a direct relationship of measured light produced (RLU) to concentration of free PSA protein in the serum sample. Serum results are expressed as ng/mL.

An active calibration curve is required for all tests. For the Access Hybritech free PSA assay, 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.

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. Prime system (pipettor, dispense, and substrate) 4 times
3. Check reagent, substrate, wash buffer, and reaction vessel status. Load any needed supplies onto the instrument. Mix reagent pack contents by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs — mix reagents by swirling gently.

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

1. Gently mix, uncap and load specimens into specimen racks, with the barcode in the open slot. Make sure there are no bubbles. Alternately, use the barcode wand to identify the specimens, and then load samples into the appropriate sample cups. Load the racks onto the instrument.
2. Each day run a 1:100 dilution of a "pool" of patient specimens to check for high dose hook. The pool result should not be higher than the highest patient result. Refer all questions to a supervisor.
3. Select the free PSA test. Note: if Total PSA is also ordered, this test can be run on the same aliquot. Testing is done in singlicate.
4. The instrument automatically calculates all results. After testing is completed, results are printed and review by the technologist. Samples with results > 20 ng/mL are diluted off-line and repeated, and results are corrected for the dilution factor. Samples with results < 0.01 ng/mL are repeated to confirm. Do not rerun samples that have sat on the Access for more than 60 minutes. Pour fresh aliquots before rerunning.
5. Remove specimens and controls. Return controls to the refrigerator and refreeze specimens.
6. 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. Specimen results are entered into the assay specific results table created from the send file corresponding to the specific sample box using Excel software (Microsoft Corporation, Redmond WA). A copy of this table 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:
 - Inspect fluidics module.
 - Check system supplies and replace as needed.
 - Clean exterior of substrate, dispense, and aspirate probes.
 - Prime pipettor, dispense, and substrate 4X.
 - Verify temperature.**Shut-down:**
 - Check waste containers, empty if needed
 - Perform special clean
2. Weekly Maintenance:
 - Change probes and clean them
 - Clean exterior of the analyzer
 - Clean upper portion of the main pipettor with alcohol wipe
 - Inspect waste filter bottle for fluid
 - Run system check
3. Periodic Maintenance to be performed by the manufacturer's service engineer.

e. Calculations

1. 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.
2. Free PSA results are reported in ng/mL and as a percentage of the total PSA.

Both total PSA and free PSA should be determined on the same serum sample. The percent free PSA (ratio) is calculated using the following formula:

$$\frac{\text{free PSA (ng/mL)}}{\text{total PSA (ng/mL)}} \times 100\% = \text{percent free PSA}$$

9. REPORTABLE RANGE OF TEST RESULTS

Free PSA results are reported to the nearest hundredth (0.01). The lowest reportable free PSA result is 0.01 ng/mL. The assay does not have a maximum reportable limit since off-line dilutions can be made to bring the concentration within the working range of the assay. The percent free percent is reported to the nearest whole number. 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 judgements 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 prepared 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 ranges for free PSA.
- 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.

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 upper reportable value is virtually unlimited. The upper limit for undiluted specimens is determined by the calibration material supplied by the manufacturer. Values exceeding this upper limit are repeated on dilution until values, prior to correction for dilution, fall between approximately 1.00-20.00 ng/mL. High samples are diluted with the zero standard.
- b. The lowest reportable value is 0.05 ng/mL. Values below this lower limit are repeated to confirm the result. If results are confirmed, report as <0.05 ng/mL and report the percentage as "NOCALC" (unable to calculate)
- c. free PSA results should be interpreted in light of the total clinical presentation of the patient, including clinical history, data from additional tests and other appropriate information. The 5 alpha-reductase inhibitor drugs, e.g. finasteride, may affect PSA levels in some patients. Other drugs used to treat benign prostatic hyperplasia (BPH) may also affect PSA levels. Care should be taken in interpreting results from patients taking these drugs.

- d. This assay does not demonstrate "high dose hook" below 20,000 ng/mL.
- e. The following substances do not interfere with the assay:
 - Hemoglobin up to 500 mg/dL
 - Bilirubin up to 20 mg/dL
 - Triglycerides up to 1500 mg/dl
 - Total protein levels of 3.8-14.1 g/dL
 - Many different drugs, see the manufacturer's kit insert for a complete list and concentrations tested.
 - No significant interference was seen in recovery studies done with a specimen with a RF of 20,000 IU/mL, a specimen with a solid phase immune complex level of 73 AHG equiv./mL, a specimen with polyclonal gamma of 3.4 g/dL, or a specimen with an alkaline phosphatase over 1000 U/L.
- g. Human anti-mouse antibodies (HAMA) may be present in samples from patients who have received immunotherapy utilizing monoclonal antibodies. Additionally, other heterophile antibodies such as human anti-goat antibodies and human antibodies reactive with murine antibodies may be present in patient samples. This assay has been formulated to minimize the effects of these antibodies on the assay. However, results on patients that are known to have such antibodies should be interpreted carefully.
- h. The concentrations of PSA in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity. Values obtained with different assay methods cannot be used interchangeably. The total PSA result used for calculation of the percent free PSA must be obtained using the Hybritech PSA method (also available on the Beckman Access).
- i. Cross reactivity with PSA-ACT was determined to be less than 1%.

13. REFERENCE RANGES (NORMAL VALUES)

Free PSA values alone are not used in patient management and do not have a reference range. The following comment is appended to results to aid in interpretation:

Among men age 50 or over with PSA values between 4.0 and 10.0 ng/mL, a free PSA of less than or equal to 10% of the total PSA is highly associated with cancer. In contrast, a free PSA greater than 25% is associated with cancer in approximately 10% of cases.

Catalona, Smith, Wolfert, et al. Evaluation of percentage of free serum prostate-specific antigen to improve specificity of prostate cancer screening. JAMA 1995; 274: 1214-1220."

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 °C during testing. After testing, the samples are stored at -70 °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 °C for no longer than 8 days. Otherwise, specimens should be stored -70 °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. The primary results include daily test results as well as stored quality control results.

The original NHANES IV ship file is copied into a template Excel file and onto the hard drive of a PC computer. After the results are entered into the database and assay results transmitted electronically. Files are stored for 6 months on a server that is backed up on a daily basis. After 6 months, the result files are transferred onto a CD along copies of original ship files and QC information.

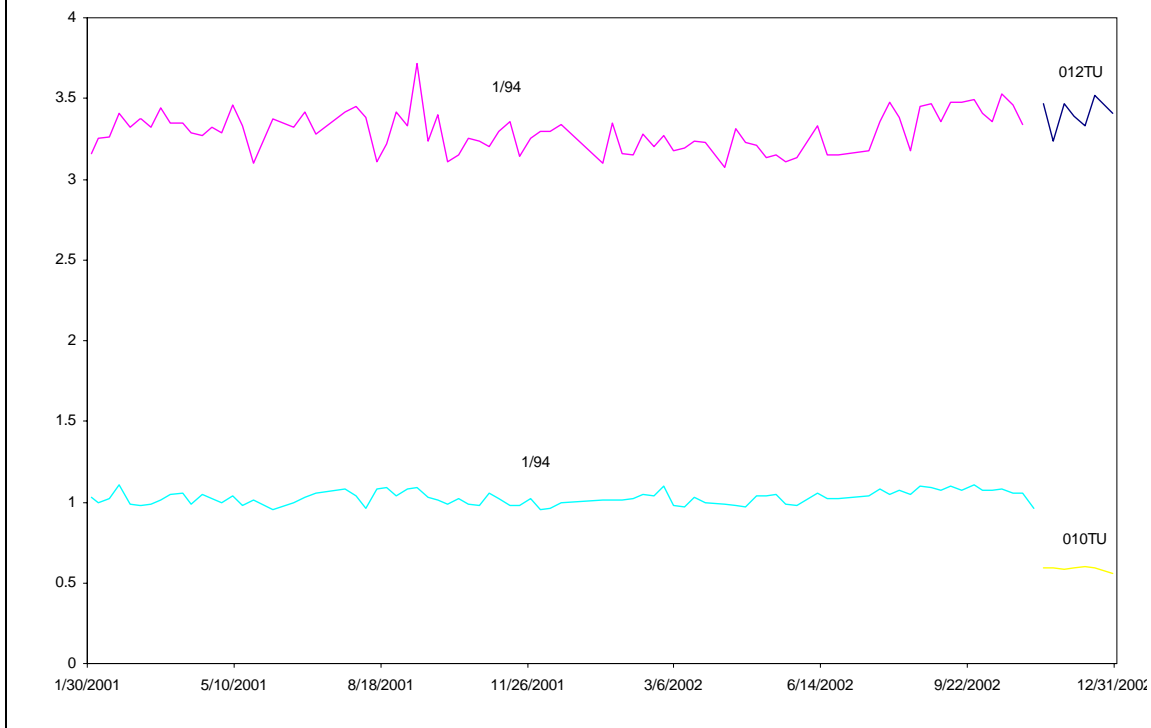
The residual serum is stored at ≤ -70 °C for 6 months after analysis, then it is returned to the NHANES Repository in Rockville, MD for long-term storage.

19. SUMMARY STATISTICS AND QC GRAPHS

Summary Statistics for Free Prostate Specific Antigen by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
1/94	83	2/2/2001	11/6/2002	3.30	0.124	3.8
1/94	83	2/2/2001	11/6/2002	1.03	0.042	4.1
012TU	7	11/13/2002	12/30/2002	3.40	0.096	2.8
010TU	7	11/13/2002	12/30/2002	0.59	0.013	2.2

2001-2002 Free Prostate Specific Antigen Quality Control



REFERENCES

1. Manufacturer Information:
 - Beckman Access Immunoassay System Operator's Guide and Reference Manual
 - Beckman Access Hybritech free PSA product insert #37210.
 - Beckman Access Hybritech free PSA Calibrators product insert #37215.
 - Beckman Access Substrate product insert #170279.
 - Beckman Access Wash Buffer product insert #170278.
2. Schacht, M.J., Garnett, J.E., and Grayhack, J. Biochemical Markers in Prostatic Cancer. Uro. Clin. No. Amer. 11:253, 1984.
3. Wang, M.C., Valenzuela, L.A., Murphy, G.P., and Chu, T.M. Purification of a Human Prostate Specific Antigen. Invest. Urol. 17:159, 1979.
4. Nadji, M., Tabei, S.Z., Castro, A., Chu, T.M., Murphy, G.P., Wang, M.C. and Morales, A.R. Prostatic-Specific Antigen: An Immunohistologic Marker for Prostatic Neoplasms. Cancer 48:1229, 1981.
5. Wang, M.C., Papsidero, L.D., Kuriyama, M., Valenzuela, L.A., Murphy, G.P., and Chu, T.M. Prostatic Antigen: A New Potential Marker for Prostatic Cancer. Prostate 2:89, 1981.
6. Frankel, A.E., Rouse, R.V., Wang, M.C., Chu, T.M. and Herzenberg, L.A. Monoclonal Antibodies to a Human Prostate Antigen. Cancer Res. 42:3714, 1982.
7. Christensson, A., Laurell, C.B., Lilja, H., Enzymatic Activity of Prostate-Specific Antigen and its Reactions with Extracellular Serine Proteinase Inhibitors. Eur.J.Biochem 194:755, 1990
8. Lilja, H., Christensson, A., Dahlen, V., Matikainen, M.T., Nilsson, O., Petterson, K., Lovgren, T., Prostate-Specific Antigen in Human Serum Occurs Predominately in Complex with alpha-1 Antichymotrypsin, Clin.Chem. 37:1618, 1991
9. Catalona, W.J., Smith, D.S., Wolfert, R., Wang, T.J., Rittenhouse, H., and Ratliff, T.L., Increased Specificity of PSA Screening through Measurement of Percent Free PSA in Serum. J.Urol (supplement), 153 (4):312a, 1995

10. Christensson, A., et al, Serum Prostate Specific Antigen Complexed to alpha-1-Antichymotrypsin as an Indicator of Prostate Cancer. J.Urol., 150:100-105, July 1993
11. Catalona, W.J., Smith, D.S., Wolfert, R.L., Wang, T.J., Rittenhouse, H.G., Ratliff, T.L., Nadler, R.B., Evaluation of Percentage of Free Serum Prostate-Specific Antigen to Improve Specificity of Prostate Cancer Screening. JAMA, 274:1214-1220, 1995