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A Comparison of Total, Free, and % Free Prostate Specific Antigen for the Serodiagnosis of Prostate Cancer in Hispanic-American and Caucasian-American Males

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Abstract: Prostate cancer is the leading non-skin cancer in males in the United States of America. The measurement of prostate specific antigen in serum has been used as a minimally invasive tool for therapeutic monitoring (remission vs. relapse and progression), screening, and, together with other tools, for diagnosis and prognosis of prostate cancer. One objective of this study was to compare two ELISA assays for prostate specific antigen and free prostate specific antigen in serum. The comparison of normal adult PSA reference intervals, predictive values, and probability of prostate cancer based on % free PSA in Hispanic-American and Caucasian-American adult males was a second objective. It was hypothesized that the Diagnostic Automation (manual) assays would be superior to those of the Beckman Access (automated) for detection of prostate cancer and that there would be a genetic bias for PSA results. Tumor marker assays were performed according to the manufacturers' directions. Assays used in this study were Total PSA (Diagnostic Automation, Inc. and Beckman Inc.) and Free PSA (Diagnostic Automation, Inc. and Beckman Inc.). A total of 1,056 samples were tested. We concluded that there was a genetic bias between Hispanic-American and Caucasian-American males. We also concluded that the manual assay was superior to the automated assay for % free PSA, but not for total PSA assays. Our hypothesis about the genetic bias and the superiority of the Diagnostic Automation for % free PSA assays was supported by the study, but our hypothesis that the Diagnostic Automation would be superior for total PSA was not supported by our findings.

Key Words: Prostate specific antigen, prostate cancer, tumor markers.

INTRODUCTION

Cancer is a prominent subject in American culture. Almost everyone in the USA either knows or will know someone who is affected by cancer in some capacity. Cancer, as it is known in our culture, is a group of diseases that are characterized by tumors. Cancer is defined by Merriam-Webster as "a malignant tumor of potentially unlimited growth that expands locally by invasion and systemically by metastasis" or "an abnormal bodily state marked by such tumors" [1]. A tumor is an abnormal growth of tissue that is known as being either malignant or benign. This tumor starts as one transformed or cancerous cell that proliferates. Generally a benign tumor is one that does not pose problems for the patient and a malignant tumor is one that invades neighboring tissues of the host, causes sickness, and at times death [2]. One characteristic growth pattern of a cancerous tumor is known as metastasis, which means to spread throughout other organs or to break off from the original point of growth and begin growing elsewhere in the body.

The word "cancer" dates back to Hippocrates (460-370 B.C.) who used the Greek terms "carcinos" and "carcinoma" to describe ulcer-forming and non-ulcer-forming tumors that he discovered. These terms mean crab and tumors probably were named this due to the finger-like growth projection that they produce. Cancer had been described before this time, but not named. In 1761, Giovanni Morgagni of Padua began performing

autopsies to find a reason for a person's death. This became the basis for the study of cancer or oncology. The famous Scottish surgeon John Hunter (1728-1793) suggested that surgeons operate on cancers that had not spread and remove tumors that could be removed. This technique flourished with the development of anesthesia a century later and practices like radical mastectomies became commonplace. Many other advances have been made assisted by the discovery of the microscope, the structure of DNA, and the different parts of the cell [3].

Cancer can potentially affect any organ or tissue. More common cancers include those affecting the prostate, breast, stomach, esophagus, pancreas, lung, and colon/rectum. According to the American Cancer Society (ACS), cancer is the second leading cause of death in the United States. An estimated 1,444,920 new cases were diagnosed in 2007 and there are projected to have been 559,650 deaths. According to the National Cancer Institute, in 2002 the prevalence of cancer was 10,146,000 people. The ACS defined cancer prevalence as "a measure of how common a cancer is" and explained that "this number is reflected by cancer incidence, which is the number of people newly diagnosed with cancer in a given time period (usually one year)." Also, cancer prevalence is "affected both by the incidence of a cancer and by how long people normally live with the disease" [4]. Prevalence is the number of all new and old cases of a disease during a particular period of time. It is expressed as a ratio of the number of new cases (numerator) per number of at risk population (denominator). These numbers are for both sexes and do not discriminate among types of cancer. Some of the most prominent cancers are breast, prostate, skin, lung, and colon/rectum. Cancer statistics, as well as the occurrence of different types of cancer vary among geographical locations.

Smoking, sexual activity, alcohol abuse, inappropriate diet, lack of physical activity, and sun exposure are some of the most common lifestyle factors that cause cancer. Other common cancer causing agents are called carcinogens which are electrophylic chemicals or other environmental factors that are responsible for causing cancer. Exposure to certain viruses may cause cancer and the presence of some diseases also contributes to the incidence of cancer within a person. Exposure to radiation can also cause cancer and there are a few hereditary cancers [5].

Some of the unique features of a cancer include cells being able to escape the host's immune system, exponential cell growth, certain biochemical markers, certain morphological properties, and molecular aberrations [5]. Cachexia (weight loss), hemorrhages, pain, palpable tumor masses, nausea, and susceptibility to other diseases are some signs and symptoms of cancer. The symptoms of cancer must be taken into account when making a proper diagnosis. These symptoms are not indicative of just one disease and tumors can be benign. Weight loss can be explained because a cancerous growth utilizes some of the nutrients that an individual ingests. Pain occurs mainly because the tumor is pressing on other tissues or nerves in the body [6].

There is not one definitive test for cancer. Some diagnostic tools that are often used in concert to detect cancer are biopsy, imaging, physical exam, chemical markers, tumor markers, nucleic acid markers, endoscopy, and cancer staging [7]. If a physician orders a blood test or urine test, it is usually to see what else is going on inside the body pertaining to electrolytes or the function of organs. Also, complete blood counts and bone marrow aspirations can be performed to look for abnormal cells. Biopsy is generally used to confirm diagnosis or to determine if a tumor is benign or malignant. Imaging techniques such as x-ray, magnetic resonance imaging (MRI), computed tomography (CT), and ultrasounds can be done to locate and examine a tumor in the body without causing the patient to endure an invasive and painful procedure. Once a cancer is diagnosed, a stage is assigned to it based upon the abnormality of the cells. The TNM system is one of the most commonly used staging systems. This system has been accepted by the International Union Against Cancer (UICC) and the American Joint Committee on Cancer (AJCC). Most medical facilities use the TNM system as their main method for cancer reporting. The TNM system is based on the extent of the tumor (T), the extent of spread to the lymph nodes (N), and the presence of metastasis (M). A number is added to each letter to indicate the size or extent of the tumor and the extent of spread. A stage one cancer has relatively normal cells while a stage five cancer has extremely abnormal cells [8]. Radiation therapy, surgery, chemotherapy, immunotherapy, hormonal therapy, and gene therapy are some forms of treatment for cancer. Cancer prevention generally involves life style changes, and preventative surgery. Life style changes such as cessation of smoking and use of other tobacco products, improved diet and exercise habits, and relief of stress often are most effective preventative measures [8]. The use of a vaccine directed against human papilloma virus (HPV), which is responsible for most of the cervical cancer cases, is an

exciting new preventative approach [5]. The prostate is an accessory male reproductive organ that is approximately the size and shape of a walnut. This organ is located in front of the rectum and just below the urinary bladder. The main function of the prostate is to store and secrete a clear, slightly alkaline (pH 7.29) fluid that constitutes 10-30% of the seminal fluid, which, along with spermatozoa, constitutes semen [5].

Prostate cancer is the second most common type of cancer seen in American men and skin cancer is the most common. However, prostate cancer causes the most cancer deaths in men [9]. The ACS estimates that during 2006 about 234,460 new cases of prostate cancer were diagnosed in the United States. Approximately 1 in 6 men will be diagnosed with prostate cancer during their lifetime, but only 1 man in 34 will die of this disease. A little over 1.8 million men in the United States are survivors of prostate cancer. While the exact causes of prostate cancer are not yet determined, there are a number of risk factors that greatly increase the chances of an individual developing prostate cancer. The risk factors are: older age, having prostate cancer in male relatives, being African- American or Caucasian, not having a healthy diet, history of gonorrhea, and lack of physical activity [9].

Vasectomy was thought to increase the risk of having prostate cancer, but this theory is not supported by empirical evidence. Symptoms usually present later in the course of the disease and include blood in the urine, burning during urination, weak or interrupted urine flow, the need to urinate frequently (especially at night), impotence, lymphatic obstruction, anemia, weight loss, pulmonary congestion, and pain in the area around the prostate (including the lower back, the pelvis and the upper thighs). Prostate specific antigen testing and digital rectal examination are the two most commonly used diagnostic tools in detecting prostate cancer [9].

It is recommended by the ACS that men begin prostate cancer screening at age 50. However, African-Americans and other men with a history of prostate cancer in the family should get tested by age 45. A biopsy is often done when a tumor is located. It is also important to note that ultrasounds can help physicians to visualize a mass or tumor [9]. Some common treatments of prostate cancer include radiation, hormone therapy, and radical removal of the prostate. Many people who have the prostate removed or the radiation therapy live for up to fifteen years after their treatment.

MATERIALS

Dilutions were prepared using the diluent supplied by the manufacturer. Statistical analysis of the results was performed using SPSS software. All procedures performed in this study were in agreement with ethical standards established by the University of Southern Mississippi (USM). Permission for the study was granted by the USM Human Subjects Protection Review Committee in accordance with Federal Drug Administration regulations (21 CFR 26, 111) and Department of Health and Human Services regulations (45 CFR Part 46).

Test samples were obtained from Memorial Hospital at Gulfport, Singing River Hospital, and Wilford Hall Medical Center (United States Air Force Base, San Antonio). The serum samples were collected using aseptic techniques by hospital employed professionals. The diagnoses of these patients were made by the attending physicians based on pathological examination. Sera were collected, separated, coded, and frozen at -20°C. Later aliquots were thawed at 37°C and assayed in a blind fashion following the manufacturers' test directions in duplicate, sample permitting, for the tumor markers.

Patients were classified as being either: a) with prostate cancer or b) without prostate cancer. Since there was incomplete information about therapeutic/drug regiments, statistical analyses included the entire patient pool (Table 1).

Table 1. Patient Classification

Number of Patients	Cancer Diagnosis
155	With Prostate Cancer
901	Without Prostate Cancer

Eight hundred and nine adult males in good health were selected and tested with no bias in the same manner as the test patients (Table 2).

Table 2. Healthy Control Subjects

Number of Patients	Ethnicity
584	Caucasian-American
184	African-American
41	Hispanic-American

The healthy control subjects consisted of 41 Hispanic-American, 584 Caucasian-American and 184 African-American adult males. These subjects were used

to generate a healthy adult reference interval/normal reference interval (NRI).

The results for the manual method reactions were read with a Beckman Coulter AD340 plate reader and all washings were done with a Stat Fax 2600 plate washer. Automated assays were performed by the hospitals before the samples were shipped and were performed using a Beckman Coulter Synchron LXi 725/ Beckman Access.

METHODS

The PSA enzyme immunoassay test kit by Diagnostic Automation, Incorporated is intended for the quantitative determination of PSA in human serum. The test is a solid phase two-site immunoassay. Rabbit anti-PSA is coated on the surface of the microtiter wells and another anti-PSA monoclonal antibody labeled with horseradish peroxidase is used as the tracer. The PSA molecules present in the standard solution or serum are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the unbound antibody-enzyme tracers are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by a colorimetric reaction. The intensity of the color formed is proportional to the concentration of PSA present in the sample [10].

All reagents and samples were allowed to reach room temperature (18-22°C) and were mixed gently before beginning the test. A data sheet with well numbers from the plate was marked with sample identification. All calibrators and controls were tested in duplicate. The assay procedure listed in Figure 1 was followed.

Figure 1. Diagnostic Automation Procedure for Prostate Specific Antigen (PSA) Enzyme Immunoassay Test Kit

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 µl of standards, specimens, and controls into the appropriate wells.
3. Dispense 100µl of zero buffer into each well.
4. Thoroughly mix for 10 seconds. It is very important to have a complete mixing in this setup.
5. Incubate at room temperature (18-22°C) for 60 minutes.
6. Remove the incubation mixture by emptying plate contents into a waste container.
7. Rinse and empty the microtiter wells 5 times with running tap or distilled water.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100µl of Enzyme Conjugate Reagent into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 60 minutes.
11. Remove the incubation mixture by emptying plate contents into a waste container.
12. Rinse and empty the microtiter wells 5 times with running tap or distilled water.
13. Strike the wells sharply onto absorbent paper to remove residual water droplets.
14. Dispense 100µl TMB solution into each well. Gently mix for 5 seconds.
15. Incubate at room temperature for 20 minutes.
16. Stop the reaction by adding 100µl of Stop Solution to each well.
17. Gently mix for 30 seconds to make sure that the blue color completely changes to yellow.
18. Using a microtiter plate reader, read the optical density at 450 nm within 20 minutes.

Important note: *The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.*

The free-PSA (f-PSA) enzyme immunoassay test kit by Diagnostic Automation, Incorporated is intended for the quantitative determination of f-PSA in human serum. The test is a solid phase two-site immunoassay. An anti-f-PSA monoclonal antibody is coated on the surface of the microtiter wells and another anti-PSA monoclonal antibody labeled with horseradish peroxidase is used as the tracer. The f-PSA molecules present in the standard solution or serum are "sandwiched" between the two antibodies. Following the formation of the coated

antibody-antigen-antibody-enzyme complex, the unbound antibody-enzyme tracers are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by a colorimetric reaction. The intensity of the color formed is proportional to the concentration of f-PSA present in the sample [11].

All reagents and samples were allowed to reach room temperature (18-22°C) and were mixed gently before beginning the test. A data sheet with well numbers from the plate was marked with sample identification. All calibrators and controls were tested in duplicate. The assay procedure listed in Figure 2 was followed.

Figure 2. Diagnostic Automation Procedure for Free Prostate Specific Antigen (f-PSA) Enzyme Immunoassay Test Kit

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards, specimens, and controls into the appropriate wells.
3. Dispense 100µl of sample diluent into each well.
4. Thoroughly mix for 10 seconds. It is very important to have a complete mixing in this setup.
5. Incubate at 37°C for 60 minutes.
6. Remove the incubation mixture by emptying plate contents into a suitable waste container.
7. Rinse and empty the microtiter wells 5 times with running tap or distilled water.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 200µl of Enzyme Conjugate Reagent into each well. Gently mix for 5 seconds.
10. Incubate at 37°C for 60 minutes.
11. Remove the incubation mixture by emptying plate contents into a suitable waste container.
12. Rinse and empty the microtiter wells 5 times with running tap or distilled water.
13. Strike the wells sharply onto absorbent paper to remove residual water droplets.
14. Dispense 100µl TMB solution into each well. Gently mix for 5 seconds.
15. Incubate at room temperature for 20 minutes in the dark.
16. Stop the reaction by adding 100µl of Stop Solution to each well.
17. Gently mix for 30 seconds to make sure that the blue color completely changes to yellow.
18. Using a microtiter plate reader, read the optical density at 450 nm within 20 minutes.

Important note: *The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.*

RESULTS

Quality control samples analyzed over a three month period were used to determine intra- and inter-assay precision (Tables 3-4). The coefficient of variation (%CV) was approximately 5% or less for all but the between-run precision for the Diagnostic Automation, which were higher at 18.30% and 19.23% for Total and %Free PSA, respectively. Serial dilutions of abnormal pool samples were used to determine the linearity of the assays (Table 5 and 6). These results indicate good linearity for the assays with all being at or above 0.9981. The minimum detectable concentration was determined by analyzing approximately 20 replicates of the diluent and establishing the mean +2SD as the cut-off value (Tables 7 and 8). Analytical sensitivities for the assays ranged from 0.000 to 0.008.

The normal reference intervals are given in Tables 9 and 10. They were slightly higher than those cited by the manufacturers' package inserts. There was significant difference between the normal reference intervals of Hispanic-American and Caucasian-American adult males for total PSA, free PSA, and % free PSA by the manual assay and for Total PSA by the automated method.

A comparison of normal adult PSA values by genetic background and by methodology is given in Tables 11-14. A comparison of normal adult PSA, free PSA, and % free PSA by methodology revealed a significant difference for free PSA and % free PSA, but no significant difference for Total PSA.

Table 3. Comparison of Diagnostic Automation and Beckman Access Assay Precision for Total PSA using Control Sera

Precision	n	$\bar{X}$ (ng/mL)	SD (ng/mL)	CV (%)
Within-Run				
Diagnostic Automation	4	3.89	0.10	2.48
Beckman Access	2	1.00	0.02	2.00
Between-Run				
Diagnostic Automation	52	3.87	0.71	18.30
Beckman Access	40	1.00	0.02	2.20

Table 4. Comparison of Diagnostic Automation and Beckman Access Assay Precision for Free PSA using Control Sera

Precision	n	$\bar{X}$ (ng/mL)	SD (ng/mL)	CV (%)
Within-Run				
Diagnostic Automation	4	2.25	0.09	4.10
Beckman Access	2	1.04	0.02	1.79
Between-Run				
Diagnostic Automation	52	2.08	0.40	19.23
Beckman Access	40	1.04	0.04	3.40

Table 5. Comparison of Diagnostic Automation and Beckman Access Assay Linearity for Total PSA

Assay	R Squared (R^2)
Diagnostic Automation	0.9981
Beckman Access	0.9996

Table 6. Comparison of Diagnostic Automation and Beckman Access Assay Linearity for Free PSA

Assay	R Squared (R^2)
Diagnostic Automation	0.9998
Beckman Access	0.9986

Table 7. Comparison of Diagnostic Automation and Beckman Access Assay Sensitivity (Analytical Sensitivity) for Total PSA

Analytical Sensitivity	n	$\bar{X}$ (ng/mL)	SD (ng/mL)	Range (ng/mL)
Assay				
Diagnostic Automation	19	0.00	0.000	0-0.000
Beckman Access	20	0.00	0.004	0-0.008

Table 8. Comparison of Diagnostic Automation and Beckman Access Assay Sensitivity (Analytical Sensitivity) for Free PSA

Analytical Sensitivity	n	$\bar{X}$ (ng/mL)	SD (ng/mL)	Range (ng/mL)
Assay				
Diagnostic Automation	20	0.00	0.000	0-0.000
Beckman Access	20	0.00	0.002	0-0.005

Table 9. Comparison of Diagnostic Automation and Beckman Access Assay Healthy Adult Reference Intervals for Total PSA

Healthy Adults	n	$\bar{X}$ (ng/mL)	SD (ng/mL)	Range (ng/mL)
Total Males				
Diagnostic Automation	808	1.67	2.86	0-7.39
Beckman Access	809	1.91	6.59	0-17.07
Hispanic-American Males				
Diagnostic Automation	28	2.56	0.59	1.38-3.74
Beckman Access	28	0.93	1.05	0-3.03
Caucasian-American Males				
Diagnostic Automation	582	1.45	2.34	0-6.13
Beckman Access	584	1.70	3.37	0-8.84

Table 10. Comparison of Diagnostic Automation and Beckman Access Assay Healthy Adult Reference Intervals for Free PSA

Healthy Adults	n	$\bar{X}$ (ng/mL)	SD (ng/mL)	Range (ng/mL)
Total Males				
Diagnostic Automation	808	0.07	0.28	0-0.63
Beckman Access	36	0.90	1.26	0-3.42
Hispanic-American Males				
Diagnostic Automation	28	0.09	0.05	0-0.19
Beckman Access	0	-	-	-
Caucasian-American Males				
Diagnostic Automation	582	0.05	0.23	0-1.02
Beckman Access	26	0.65	0.43	0-1.51

Table 11. Comparison of Normal Adult Total PSA Values by Genetic Background

Total PSA	n	$\bar{X}$ (ng/mL)	SD (ng/mL)	Probability
Diagnostic Automation				
Hispanic-American Males	28	2.56	0.59	0.000*
Caucasian-American Males	582	1.45	2.34	
Beckman Access				
Hispanic-American Males	28	0.93	1.05	0.003*
Caucasian-American Males	584	1.70	3.37	

*p =< 0.05

Table 12. Comparison of Normal Adult Free PSA Values by Genetic Background

Free PSA	n	$\bar{X}$ (ng/mL)	SD (ng/mL)	Probability
Diagnostic Automation				
Hispanic-American Males	28	0.09	0.05	0.003*
Caucasian-American Males	582	0.05	0.23	
Beckman Access				
Hispanic-American Males	0	-	-	-
Caucasian-American Males	26	0.64	0.43	

*p =< 0.05

Table 13. Comparison of Normal Adult % Free PSA Values by Genetic Background

% Free PSA	n	$\bar{X}$ (ng/mL)	SD (ng/mL)	Probability
Diagnostic Automation				
Hispanic-American Males	28	3.52	0.85	0.003*
Caucasian-American Males	582	3.45	3.51	
Beckman Access				
Hispanic-American Males	0	-	-	-
Caucasian-American Males	26	37.6	52.9	

*p =< 0.05

Table 14. Comparison of Normal Adult PSA Values by Methodology (paired t-test)

Assay Method	n	$\bar{X}$ (ng/mL)	SD (ng/mL)	Probability
TOTAL PSA | Assay				
Diagnostic Automation	807	1.67	2.86	0.167
Beckman Access	807	1.91	6.59	
Free PSA | Assay				
Diagnostic Automation	36	0.29	0.87	0.000*
Beckman Access	36	0.90	1.26	
% Free PSA | Assay				
Diagnostic Automation	31	3.55	5.59	0.000*
Beckman Access	31	19.06	9.01	

*p =< 0.05

Table 15. Predictive Values of Total PSA for Prostate Cancer in 1056 Patients

Assay Method	Sensitivity (%)	Specificity (%)	PV (+) (%)	PV (-) (%)	Efficiency (%)	Cut-Off (ng/mL)
TOTAL Males						
Diagnostic Automation	10.32	93.11	20.51	85.77	80.95	4.00
Beckman Access	18.71	87.57	20.57	86.23	77.46	4.00
Hispanic-American						
Diagnostic Automation	-	100.00	-	100.00	-	4.00
Beckman Access	-	86.00	-	100.00	-	4.00
Caucasian-American						
Diagnostic Automation	6.06	93.09	11.76	86.70	81.60	4.00
Beckman Access	14.14	87.60	14.74	87.06	77.93	4.00

Table 16. Predictive Values of % Free PSA for Prostate Cancer in 1056 Patients

Assay Method	Sensitivity (%)	Specificity (%)	PV (+) (%)	PV (-) (%)	Efficiency (%)	Cut-Off (ng/mL)
TOTAL Males						
Diagnostic Automation	97.25	4.27	13.23	91.18	16.41	25.00
Beckman Access	80.00	33.33	5.13	97.37	35.34	25.00
Hispanic-American						
Diagnostic Automation	-	100.00	-	100.00	-	25.00
Beckman Access	-	-	-	-	-	25.00
Caucasian-American						
Diagnostic Automation	98.15	3.13	11.74	94.12	14.16	25.00
Beckman Access	75.00	33.75	5.36	96.43	35.71	25.00

Cutoffs to determine normal (negative) and abnormal (positive) test results used those cited by the manufacturers (Tables 15 and 16). Using the cut off values established by the manufacturers, we obtained diagnostic sensitivities of <50% by both methods for Total PSA.

Diagnostic sensitivity of a test is the proportion of individuals with the disease who test positively with the test. The diagnostic sensitivities for % free PSA were excellent. Diagnostic sensitivities were not calculated for the Hispanic-American males due to the paucity of positive samples. Diagnostic specificities were, however, excellent for both total and % free assays using the manual method and for total PSA using the automated method. Diagnostic specificity of a test is the proportion of individuals without the disease who test negatively with the test. The other predictive values were as expected. Predictive value (+) is the fraction of positive tests that are true positives. Predictive value (-) is the fraction of negative tests that are true negatives. Diagnostic efficiency is the fraction of all test results that are either true positives or true negatives.

DISCUSSION & CONCLUSION

Based on the results of our study, the hypothesis that there would be a genetic bias between Hispanic-American and Caucasian-American men was accepted. In contrast, the hypothesis that the manual assay would be superior to the automated assay was rejected for total PSA assays, but supported for % free PSA assays. Analytical parameters were acceptable for all the assays. As stated previously, the normal reference intervals we determined were slightly higher than those cited by the manufacturers' package inserts. There was significant difference between the normal reference intervals of Hispanic-American and Caucasian-American adult males for total PSA, free PSA, and % free PSA by the manual assay. This is the first report of a comparison of PSA healthy (normal) adult reference intervals for Hispanic-American males and Caucasian-American males. It is noteworthy that there was a statistically significant difference between the two groups, leading one to believe that one should use a modulated reference interval when diagnosing and monitoring Hispanic-American males. A comparison of normal adult PSA reference intervals by methodology showed significant difference for free PSA and % free PSA, but not total PSA. The Beckman Access results were typically slightly higher than those from the Diagnostic Automation. This would indicate a need for consistent use of one or the other method when diagnosing and performing therapeutic monitoring on individual patients.

Using the cut off values established by the manufacturers, we obtained diagnostic sensitivities of <50% for Total PSA by both methods. While our values were in line with those of other researchers, they were still disappointing. It is speculated that this may be due to inclusion of patients with prostate cancer who may have been diagnosed and were undergoing treatment. Alternatively, the patients may have been diagnosed earlier in the course of the disease.

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