Method Comparison Studies for Prostate Specific Antigen and Unconjugated Estriol Immunoassays

MARY E KOENN, BONIFACE V NDAH

OBJECTIVE: Method comparison studies were performed in order to move a semi-automated prostate specific antigen (PSA) immunoassay and a manual unconjugated estriol (uE_3) immunoassay to an automated chemistry immunoassay analyzer. The results of the two method comparison studies are compared.

DESIGN: Serum samples collected on patients with physician orders for PSA or uE_3 were assayed by both methods. PSA samples were assayed on a Hybritech Tandem Photon ERA and on two Beckman Coulter Access instruments. UE_3 samples were assayed by RIA and on two Beckman Coulter Access instruments. Linear regression analysis was performed on both sets of data and within-run precision and dilution studies were performed on the PSA Access method.

SETTING: Clinical chemistry laboratory, West Virginia University Hospitals Inc, Morgantown WV.

RESULTS: PSA linear regression analysis for the two methods (ERA and Access 1) were y = 1.0008x + 0.0393, r = 0.9976, SE = 0.1319, n = 37 and (ERA and Access 2), y = 1.0019x + 0.0486, r = 0.9964, SE = 0.1632, n = 37. Within-run precision studies for both Access instruments produced acceptable coefficient variations and dilution study results were in PSA reportable range. uE_3 linear regression analysis for the two methods (RIA and Access 1) were y = 1.4105x - 0.3741, r = 0.8696, SE = 0.8330, n = 33 and (RIA and Access 2) were y = 1.315x - 0.2292, r = 0.8643, SE = 0.7964, n = 33.

CONCLUSION: The results of the method comparison studies for PSA were acceptable and the automated PSA immunoassay method was adopted. The results of the uE_3 comparison studies did not show good correlation; the automated method was not adopted.

ABBREVIATIONS: CV = coefficient of variation; MoM = multiple of the median; PSA = prostate specific antigen; r = correlation coefficient; RIA = radioimmunoassay; SE = standard error; uE_3 = unconjugated estriol.

INDEX TERMS: immunoassay; method comparison studies; prostate specific assay; unconjugated estriol.

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Among American men, prostate cancer is the most common malignancy and the second leading cause of cancer mortality. According to autopsy studies, approximately nine million American men may now have prostate cancer.[1] In most patients, the malignancy grows slowly, resulting in different grades of tumor confined to the prostate gland and at this stage, often still curable. Rapid growth and metastasis beyond the prostate are seen in some patients creating a less favorable long-term survival. Early detection along with local treatment is necessary in the management of this disease.[2]

Although controversial, prostate specific antigen (PSA) determination, in conjunction with digital rectal examination (DRE) are currently recommended for screening all men age 50 and older.[1,3] Other uses of PSA levels include pretreatment staging and post-treatment monitoring.[2,4] Screening for prostate cancer with PSA has significantly increased the volume of PSA tests performed by clinical laboratories. A marked increase in reported incidence of prostate cancer has also occurred. Since PSA is found in benign prostatic hyperplasia and inflammatory conditions of the prostate, interpretation of results should be used with DRE or ultrasound.[3]

UNCONJUGATED ESTRIOL

Estrogens are primarily secreted by the ovarian follicles and the corpus luteum in the non-pregnant females; in pregnancy, the major source is the placenta. The primary estrogen secreted by the ovary is estradiol, whereas that of the placenta is estriol. The placenta forms estriol by sequential desulfurylation and aromatization of the androgen,

dehydroepiandrosterone sulfate (DHEA-S).[5] Approximately 9% of the estriol remains unconjugated and is tightly bound to sex hormone-binding globulin. The majority is conjugated to glucuronates and sulfates in the maternal liver permitting renal clearance.[6]

Maternal serum testing is a well-established screening procedure for the detection of congenital anomalies. Measurement of unconjugated estriol (uE_3) in the third trimester was first used to assess fetal well being.[6]

During the second trimester, serum levels of uE_3 are decreased in fetal Down syndrome.[6] A combination of uE_3, alpha fetoprotein (AFP) and chorionic gonadotropin (CG) as a triple marker is now used for fetal screening at 16 weeks gestation for Down syndrome. Like uE_3, AFP is decreased in Down syndrome, while CG is increased.[5,6] Reporting the triple screen results in conventional units and as multiple of the median (MoM) identifies about 5% of patients with increased risk of Down syndrome and detects approximately 60% of actual cases of Down syndrome.[6] Low levels of uE_3 are also associated with pregnancy-induced hypertension, miscarriage, intrauterine growth restriction, and intrauterine fetal death.[7]

MATERIALS AND METHODS

The clinical chemistry laboratory performed method validation studies to move a semi-automated PSA immunoassay and a manual uE_3 immunoassay to an automated chemistry immunoassay analyzer. All study assays were performed according to manufacturers' recommendations; calibrators, standards, and controls for assays were utilized according to laboratory approved procedures.

Semi-automated PSA

The Hybritech Tandem Photon ERA (Beckman Coulter Inc, Fullerton CA), a two-site immunometric (sandwich) semi-automated assay, quantitates PSA in serum samples. The capture monoclonal antibody is against a unique site on the PSA molecule, and the other antibody, enzyme-labeled, is directed against a different PSA molecule site. The assay is completed with the addition of the photon enzyme substrate and the color formed is measured in the PHOTON ERA instrument. The quantity of color detected is proportional to the concentration of PSA in each sample.

Manual uE_3

The Ultra-Sensitive uE_3 (Diagnostic Systems Laboratories Inc, Webster, TX) is a competitive binding manual radioimmunoassay. The antigens, ^{125}I-labeled uE_3 and patient serum sample uE_3, compete for a fixed number of antibody (rabbit anti-uE_3) binding sites. After incubation and formation of antigen-antibody complexes, a double antibody system separates free antigen from the antibody-bound antigen. For this separation, a goat anti-rabbit precipitating reagent is added and centrifugation completes the separation. A Gamma Counter (Packard Instrument Co, Downers Grove IL) measures the amount of bound ^{125}I-labeled uE_3; the count is inversely proportional to the concentration of uE_3 present in the patient sample.

Automated immunoassay analyzer methods

For the comparison studies, both sets of patient samples were assayed on each of two Beckman Coulter Access Immunoassay analyzers (Beckman Coulter Inc, Fullerton CA). The Access is a chemiluminescent immunoassay measurement system using paramagnetic particles coated with antibody to the analyte of interest and the chemiluminescent substrate, dioxetane phosphate. Alkaline phosphatase-labeled complexes react with the substrate creating a chemical reaction and a source of energy to excite the dioxetane substrate. The light emitted is quantitated in the analyzer luminometer.

The PSA Access assay is a two-site immunometric assay using the same Hybritech antibodies as the Photon ERA method. The anti-PSA coating the paramagnetic particles captures the PSA molecule and a second antibody labeled with alkaline phosphatase completes the sandwich by binding to a different antigenic site on the PSA molecule. Addition of the dioxetane substrate initiates the chemiluminescent reaction and the amount of light measured in the luminometer is proportional to the concentration of PSA in the sample.

The uE_3 Access assay is a competitive binding immunoenzymatic assay; serum uE_3 and alkaline phosphatase-labeled uE_3 compete for a fixed number of uE_3 antibodies. The antigen-antibody complexes attach to the paramagnetic particles and the enzyme-labeled complexes initiate the chemiluminescent reaction. The amount of light quantitated in the luminometer is inversely proportional to concentration of uE_3 in patient samples.

Split-sample comparative studies, 40 samples for PSA and 33 for uE_3 studies, were assayed with present laboratory methods and then on the Access 1 and Access 2 instruments. Method comparison studies were performed to derive the linear regression equation, correlation coefficient (r), and standard error (SE). Further statistical testing of within-run precision and linear range completed the PSA evaluation.

RESULTS

PSA

Linear regression analysis showed good agreement between the semi-automated Hybritech Photon ERA PSA and the automated Access 1 and Access 2 immunoassay measurements. Three high PSA results (outliers) were discarded. The linear regression plot and equation for ERA and Access 1 is shown in Figure 1. Analysis resulted in r = 0.9976, SE = 0.1319 for n = 37. See Table 1, 0-10 µg/L. Comparison analysis for the ERA and Access 2 were very similar, r = 0.9964, SE = 0.1632 for n = 37. See Figure 2, 0-10 µg/L and Table 2.

Figure 1. Comparison of Beckman Coulter Access 1 PSA and ERA PSA by linear regression analysis

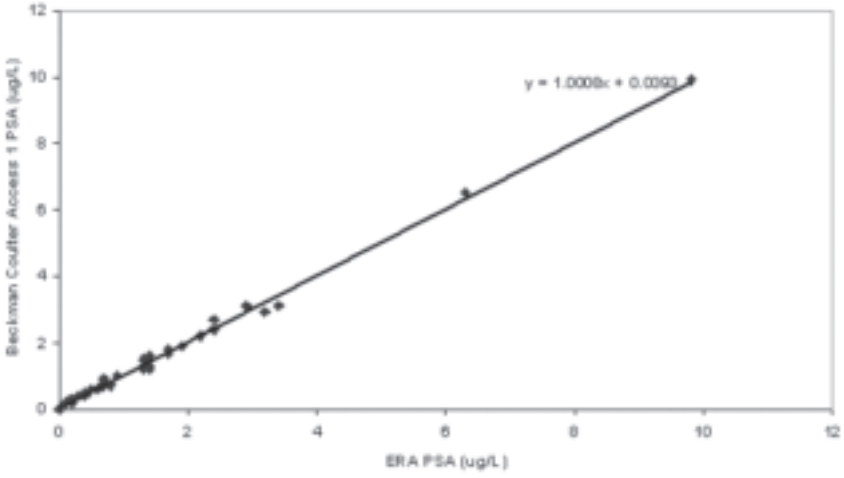
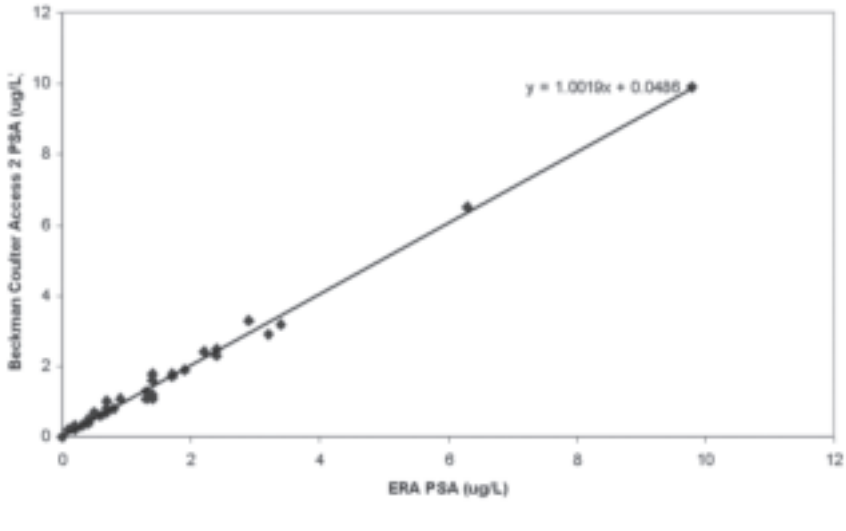


Figure 2. Comparison of Beckman Coulter Access 2 PSA and ERA PSA by linear regression analysis



Within-run precision studies for both analyzers resulted in acceptable coefficient of variations (CV), Access 1, CV = 1.7 and Access 2, CV = 1.9. Dilution studies to assess the method linearity showed results within the PSA reportable range.

uE_3

Regression analysis for uE_3 did not display comparable agreement. Figure 3 displays the regression plot and equation for RIA and Access 1 assays, r = 0.8696, SE = 0.8330 for n = 33. (Table 3) Similar plots and statistics resulted with RIA and Access 2 (Figure 4), r = 0.8643, SE = 0.7964 for n = 33 (Table 4) .

DISCUSSION

PSA

Because of the good agreement resulting from the method comparison and the discontinuance of the Hybritech reagents for the Photon ERA, the PSA testing was moved to the Access analyzers. Use of the same antibodies by both methods contributed to the good correlation.

Assaying PSA specimens on an automated analyzer provides significant advantages for the chemistry laboratory. The Access, a random-access immunoassay analyzer, reduces the hands-on labor time and is interfaced to the laboratory information system, all resulting in faster turnaround time for result reporting.[3] The laboratory has performed all PSA tests on the Access instruments for the past 14 months and proficiency testing has been satisfactory.

The effect of the concentration range on the r and SE can be explained with the data from this PSA method study. Including all 40 sample results increases the concentration range from 0-100 µg/L. For the Access 1, this improves the r from 0.9976 to 0.9996, but also increases the SE from 0.1319

to 0.5870. Likewise, using samples with a concentration range 0-4 μg/L, decreases the r to 0.9905, but improves the SE to 0.1264 (Table 1). See Table 2 for similar results for Access 2.

uE_3

The correlation coefficients resulting from the uE_3 comparisons for both instruments were less than 0.99. In an attempt to expand the concentration range, additional samples would have had to be collected; otherwise, a different kind of statistical analysis would have been needed to complete the evaluation. The laboratory decided that neither of these approaches was justifiable given the time that must be expended and its new direction. The differences in results between the two methods would cause further complications incorporating uE_3 results in the MoM. Also, at this time the laboratory began an investigation in possible adoption of a different automated immunoassay analyzer.

Figure 3. Comparison of RIA uE3 and Beckman Coulter Access 1 uE3by linear regression analysis

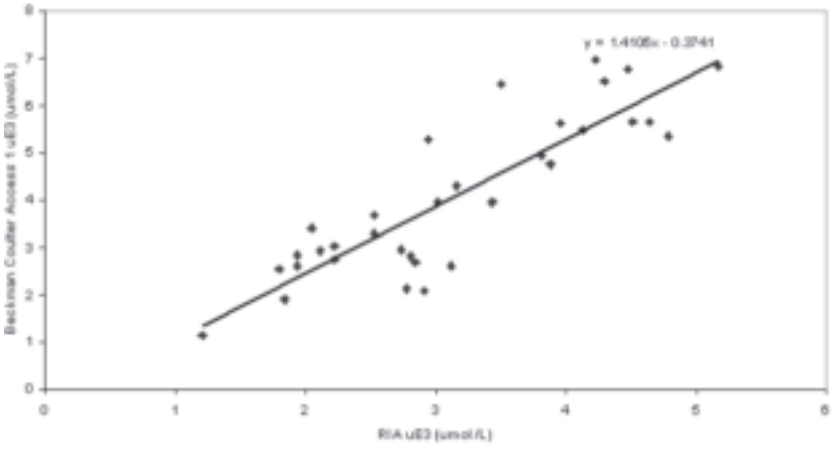
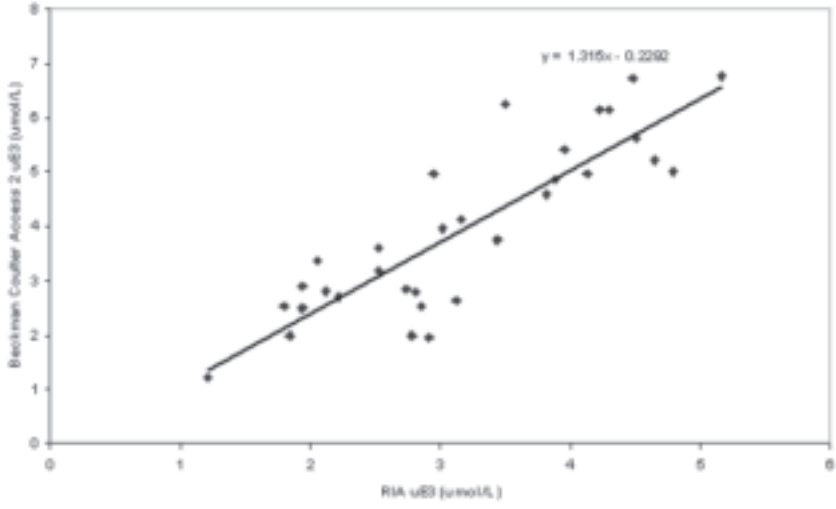


Figure 4. Comparison of RIA uE3 and Beckman Coulter Access 2 uE3by linear regression analysis



CONCLUSION

The analysis showed good agreement between the semi-automated Hybritech Photon ERA PSA and the automated Beckman Coulter Access 1 and Access 2 immunoassay measurements. The Beckman Coulter Access PSA method was adopted by the chemistry laboratory. On the other hand, the RIA and the Beckman Coulter Access methods for measuring uE_3 for this set of data did not demonstrate good agreement and the Beckman Coulter Access uE_3 was not adopted.

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Table 1. Comparison of Photon ERA PSA to Access 1 PSA

Concentration range, µg/L	n	Mean, ng/mL: ERA	Access	r	Intercept	Slope	SE
0-4	35	1.1	1.1	0.9905	0.0800	0.9584	0.1264
0-10	37	1.5	1.5	0.9976	0.0393	1.0008	0.1319
0-105	40	7.0	6.7	0.9996	0.1629	0.9374	0.5870

Table 2. Comparison of Photon ERA PSA to Access 2 PSA

Concentration range, µg/L	n	Mean, µg/L: ERA	Access	r	Intercept	Slope	SE
0-4	35	1.1	1.1	0.9847	0.0811	0.9678	0.1629
0-10	37	1.0	1.5	0.9964	0.0486	1.0019	0.1632
0-105	40	7.0	7.0	0.9998	0.1120	0.9958	0.4405

Table 3. Comparison of RIA uE_3 to Access 1 uE_3

Concentration range, µmol/L	n	Mean, µmol/L RIA	Access	r	Intercept	Slope	SE
1-7	33	3.14	4.05	0.8696	-0.3741	1.4105	0.8330

Table 4. Comparison of RIA uE_3 to Access 2 uE_3

Concentration range, µmol/L	n	Mean, µmol/L RIA	Access	r	Intercept	Slope	SE
0-7	33	3.14	3.90	0.8643	-0.2292	1.3150	0.7964

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