μg/L cutoff was determined monthly using data obtained from daily analysis of samples with target concentrations of 240 and 360 μg/L. Representative (most recent) CVs at these concentrations were as follows: 3.8% and 3.1% for codeine, 3.9% and 3.2% for morphine, 3.3% and 3.6% for hydrocodone, 3.2% and 3.9% for hydromorphone, and 4.4% and 4.5% for oxycodone, respectively.

Possible cross-interference with the quantification of codeine, morphine, hydrocodone, hydromorphone, and oxycodone was assessed by supplementing urine samples with 5000 μg/L oxymorphone and norcodeine (the two most likely interfering substances) in the presence of codeine, morphine, hydrocodone, hydromorphone, and oxycodone at 300 and 120 μg/L. In all cases, quantification of the analyte of interest was not affected by the presence of the potential interfering substance. Additionally, no cross-interference between the five opiates of interest at concentrations up to 10 000 μg/L was observed. Large concentrations of oxymorphone can interfere with the *m/z* 268 qualifier ion of morphine, but this does not interfere with the quantification of morphine.

The limit of detection (LOD) and limit of quantification (LOQ) determined by duplicate analysis of serially diluted samples were equal for each of the five analytes. The LODs and LOQs were 60 μg/L for codeine, hydrocodone, and hydromorphone; 90 μg/L for oxycodone; and 120 μg/L for morphine. The criteria for both LOD and LOQ included acceptable chromatography and acceptable ion ratios. Quantification within 20% of the target concentration was required for the LOQ but not the LOD.

In conclusion, we present a method that allows the simultaneous quantification of codeine, morphine, hydrocodone, hydromorphone, and oxycodone at concentrations from a minimum of 60–120 μg/L (LOQ for each opiate) to a maximum of 5000 μg/L for codeine, 8000 μg/L for morphine, 6000 μg/L for hydrocodone, and 3000 μg/L for hydromorphone and oxycodone. The method demonstrates acceptable precision and lack of cross-interference and interference from other opiates, uses a relatively small sample volume (2.0 mL), and has an analysis time of ~6.5 min. This method has been used in the laboratory (Memphis, TN) for the analysis of >3500 samples in a 4-month period and has been found to be reliable as demonstrated by calibrator and control reproducibility and the absence of interference.

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Hybritech Total and Free Prostate-specific Antigen Assays Developed for the Beckman Coulter Access Automated Chemiluminescent Immunoassay System: A Multicenter Evaluation of Analytical Performance, *Robert J. Laffin,[1] Daniel W. Chan,[2] Milenko J. Tanasijevic,[3] George A. Fischer,[3] Wayne Markus,[4] Joan Miller,[1] Pat Matarrese,[1] Lori J. Sokoll,[2] Debra J. Bruzek,[2] Julie Eneman,[3] Janice Nelson,[4] Kurtis R. Bray,[5] Jay Huang,[5] and Kathleen G. Loveland[5]**

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Previously reported clinical trials have shown that measurement of the different forms of prostate-specific antigen (PSA) *(1)* is useful in the differentiation of prostate cancer from benign prostatic conditions. Men with prostate cancer tend to have lower percent free PSA (%FPSA) values than men with benign disease *(2–4)*; %FPSA is the ratio of free PSA to total PSA times 100%.

Beckman Coulter, Inc. (Fullerton, CA) has developed the Access Hybritech PSA and Hybritech PSA assays for use on the automated Access Immunoassay System. The assays are two-site immunoenzymatic ("sandwich") assays that use mouse monoclonal antibody in alkaline phosphatase conjugate and paramagnetic particles coated with a second mouse monoclonal antibody. After unbound particles are removed by washing, a chemiluminescent substrate, Lumi-Phos 530,[6] is added to produce

[6] Lumi-Phos 530 is a trademark of Lumigen, Inc.

Table 1. Regression analysis for Access Hybritech PSA vs Tandem PSA and for Access Hybritech free PSA vs Tandem free PSA for four laboratories.

Lab	Assay	Deming slope	*y*-Intercept	*r*	Samples, Range n	Samples, Range μg/L
Access Hybritech PSA vs Tandem PSA						
1	Tandem-E PSA	0.989	0.53	0.993	84	0.1–97
2	Tandem-E PSA	1.099	−0.14	0.996	110	0.3–33
3	Tandem-E PSA	1.063	0.08	0.997	87	0.1–16
4	Tandem-R PSA	1.145	−0.25	0.996	119	0.1–100
All 4	Tandem PSA	1.074	0.19	0.993	400	0.1–100
Access Hybritech free PSA vs Tandem free PSA						
1	Tandem-R FPSA	1.019	−0.01	0.989	80	0.05–12
2	Tandem-R FPSA	0.980	−0.01	0.987	110	0.05–5.4
3	Tandem-R FPSA	0.967	0.05	0.991	82	0.05–4.9
4	Tandem-R FPSA	1.071	−0.05	0.989	119	0.05–19
All 4	Tandem FPSA	1.047	−0.04	0.989	391	0.05–19

light directly proportional to the amount of analyte in the sample as determined from a stored calibration curve. Calibrators are assigned up to 150 μg/L PSA and 20 μg/L FPSA.

Analytical performance of the new assays was evaluated at four clinical laboratories located in the United States and at the manufacturer's laboratories. Proficiency of the Access instruments and operators was demonstrated by assaying an identical panel of 18 human samples. Serum aliquots were stored at −70 °C, shipped frozen, and tested in duplicate over 2 days on both the Access and the reference Tandem methods. Proficiency panel results from each laboratory were analyzed by Deming regression and compared with the values assigned by the manufacturer. Each laboratory achieved results within 10% of the target values before proceeding. For the remainder of the study, Lyphochek Immunoassay Plus Control[7] and Access QC control with ranges assigned for the Access instrument for each assay were used daily to verify calibration of individual instruments and accurate sample recovery from stored calibration curves. Controls spanning the range of each assay recovered without trending and within the expected 2 SD range at the beginning and end of the 28-day stored assay calibration interval on each instrument. Consistent recovery of controls verified stable systems.

As recommended in NCCLS guideline EP5 *(5)*, we determined within-run, between-run, and total imprecision for four lots of reagent (two PSA and two FPSA), using commercial controls on one instrument in each laboratory. Among the four laboratories, the overall CV (the root mean square of the median variances) across the range of the PSA assay was 3.9% (range, 1.8–6.7%); for the FPSA assay, it was 3.8% (range, 2.5–6.0%).

The detection limit was calculated as the concentration corresponding to the mean response (relative light units) plus 2 SD of 10 replicates of the zero calibrator when read from the stored calibration curve derived from the same reagent lot (target detection limits: ≤0.008 μg/L PSA and ≤0.005 μg/L FPSA). Across laboratories using two reagent lots, the detection limit for PSA was 0.005 μg/L (range, 0.003–0.008 μg/L) and for FPSA was 0.002 μg/L (range, 0.001–0.005 μg/L). Secondly, at three laboratories, human sera (PSA <2.5 μg/L and FPSA <0.25 μg/L) were diluted in PSA sample diluent, and the within-run CVs of duplicate analyses of each dilution were calculated. For these three laboratories, the mean lowest concentration contributing to a plotted straight line of the dilutions and with CV <20% for the replicates (n = 2) was 0.007 μg/L for PSA and 0.005 μg/L for FPSA.

To verify that an assay does not give falsely low results in the presence of the very high concentrations of PSA or FPSA that may be present in human samples (antigen excess or hook effect), aliquots of the zero calibrator containing PSA concentrations of 19–625 000 μg/L and FPSA concentrations of 5–160 000 μg/L were prepared by the manufacturer. The instrument signal was plotted vs concentration for three lots of each assay to determine the point where excess antigen falsely read within the calibration range. The Access Hybritech PSA assay did not demonstrate antigen excess up to 50 000 μg/L PSA, and FPSA did not hook until >20 000 μg/L.

To demonstrate linearity, 15 samples with increased PSA (65–128 μg/L) and 7 samples with increased FPSA (6–12 μg/L) were diluted and assayed, using two reagent lots for each assay. The expected results vs observed results were analyzed by Deming regression. The target dilution linearity was a regression slope of 0.90–1.10. The slope of the Deming regression for observed PSA concentrations vs expected concentrations on the 15 samples ranged from 0.99 to 1.02 (r >0.99). The slope of the Deming regression for observed FPSA concentrations vs expected concentrations of the seven samples varied from 0.98 to 1.01 (r >0.99).

As recommended in NCCLS guideline EP7 *(6)*, we tested serum for potential interference from endogenous

[7] Lyphochek Immunoassay Plus Control is a trademark of Bio-Rad Laboratories.

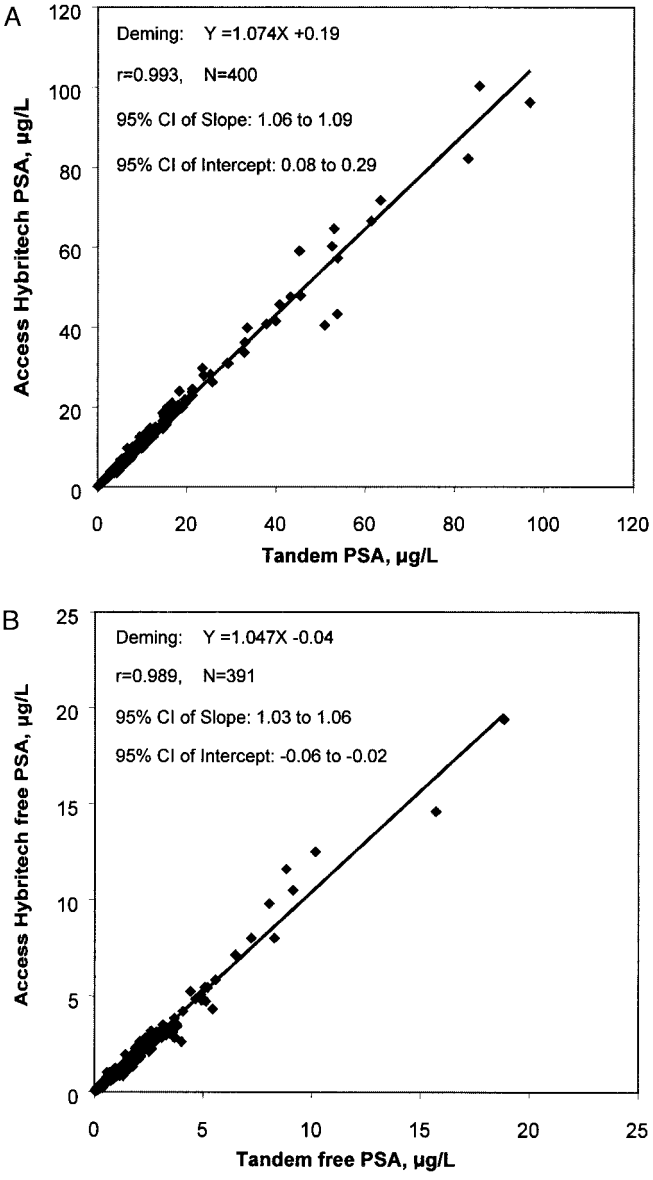

Fig. 1. Deming regression analysis of the combined multicenter data for Access Hybritech PSA vs Tandem PSA (*A*) and Access Hybritech free PSA vs Tandem free PSA (*B*).

(*A*), the Deming equation for Access Hybritech PSA vs Tandem PSA across four laboratories is: $y = 1.074x + 0.19$; $r = 0.993$; n = 400. (*B*), the Deming equation for Access Hybritech free PSA vs Tandem free PSA across four laboratories is: $y = 1.047x - 0.04$; $r = 0.989$; n = 391. *CI*, confidence interval.

substances in blood and 33 drugs frequently used by a population of men >50 years of age, and none interfered in the assay.

PSA and FPSA results from serum samples with the new assays were compared with results obtained by comparison methods: Tandem-R PSA at one laboratory, Tandem-E PSA (on the Photon® ERA) at three laboratories, and Tandem-R *free* PSA at all laboratories. Specimens for method comparisons were processed by each laboratory by routine methods within 3 h after blood was collected. Excess serum was stored frozen at −20 °C. Serum frozen for longer than 1 month was stored at −70 °C *(7, 8)*. All identifying and demographic information was removed from these leftover sera in accordance with local Institutional Review Board policies. No results from investigational reagents were reported to physicians. Method comparisons were analyzed by Deming regression for results falling within the range of each assay. Table 1 shows results by assay for each laboratory and for all laboratories combined. Fig. 1A shows the overall Access Hybritech PSA vs Tandem equation: $y = 1.074x + 0.19$ (slope range, 0.989–1.145; n = 400). Fig. 1B shows the overall equation of Access Hybritech free PSA vs Tandem free PSA: $y = 1.047x - 0.04$ (slope range, 0.967–1.071; n = 391). Because controls on the instruments in each laboratory consistently recovered within 2 SD of one another throughout the study and the overall imprecision of the Access system was <4%, differences in method comparison results were attributed to the differences in distribution of PSA concentrations in the different patient populations and variations in each individual laboratory environment.

Because the data for these specimens left over from routine testing did not include clinical or demographic information, %FPSA could not be interpreted with respect to diagnosis. However, comparison of %FPSA from four sites derived from the Access Hybritech methods and the Tandem reference methods analyzed by Deming method showed an overall regression of Access (y) assays vs Tandem (x) of: $y = 1.01x - 0.01$ ($r = 0.933$; n = 391).

To assess the ability of the Access Hybritech PSA assay to recover each PSA form [FPSA and PSA complexed with α_1-antichymotrypsin (PSA-ACT)] equally, four sets of samples with constant PSA concentrations (4, 7, 15, and 30 µg/L) and increasing proportions of FPSA (0%, 25%, 50%, 75%, and 100%) were prepared with purified, characterized, and fractionated FPSA and PSA-ACT *(9)*. At each PSA concentration, the slope of the PSA results was near zero (0.002, 0.010, 0.013, and 0.028), which indicates that the Access Hybritech PSA assay is unaffected by the proportions of FPSA in the sample. The average molar response ratio (100% FPSA/0% FPSA) for the Access Hybritech PSA assay was 1.11. These data demonstrate that the Access Hybritech PSA assay is equimolar (nonskewed). For 391 human samples in the multicenter study, the ratio of the Access Hybritech PSA assay over the Tandem PSA was plotted vs %FPSA in each sample. Again, the slope of the data was near zero ($y = 0.09x$), indicating that the PSA results from both assays responded the same in regard to the concentration of FPSA and PSA-ACT in the sample. Overestimation of true PSA recovery by a "skewed" assay can lead to unnecessary prostate biopsies, whereas underestimation can lead to delayed diagnosis. This is important because previous work has shown that the concentrations of FPSA and total PSA in a given specimen determined with assays from different manufacturers can vary widely because of differences in assay methods and reagent specificity *(10–12)*. Therefore, values obtained with different manufacturers' assays cannot be used interchangeably.

In conclusion, these automated chemiluminescent Access Hybritech PSA and free PSA assays supply information to the physician equivalent to that provided by the Hybritech Tandem manual assays. Both systems use the

same antibody pairs, and each assay is approved by the Food and Drug Administration for the same clinical indications. This multicenter evaluation shows that the performance of the automated Access Hybritech PSA and free PSA assays is analytically specific, sensitive, linear, accurate, and precise.

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Congenital Disorder of Glycosylation Ia with Deficient Phosphomannomutase Activity but Normal Plasma Glycoprotein Pattern, *Thierry Dupre,[1] Maryvonne Cuer,[1] Sandrine Barrot,[1] Anne Barnier,[1] Valérie Cormier-Daire,[2] Arnold Munnich,[2] Geneviève Durand,[1,3] and Nathalie Seta[1,4*]* ([1] Laboratoire de Biochimie A, Hôpital Bichat, 75877 Paris Cédex 18, France; [2] Service de Génétique Médicale, INSERM U393 Hôpital Necker, 75015 Paris, France; [3] Faculté de Pharmacie, Université Paris XI, 92296 Châtenay-Malabry Cédex, France; [4] Faculté de Pharmacie, Université Paris V, 75006 Paris, France; * address correspondence to this author at: Laboratoire de Biochimie A, Hôpital Bichat-Claude Bernard, 46, rue Henri Huchard, 75877 Paris Cédex 18, France; fax 33-1-40-25-88-21, e-mail nathalie.seta@bch.ap-hop-paris.fr)

Congenital disorders of glycosylation [CDG; previously carbohydrate-deficient glycoprotein syndrome *(1)*] represent a newly delineated group of inherited diseases *(2)*. The CDG are now clearly classified in two groups including subgroups. CDG I, by far the most common type with >300 patients described in the literature, is characterized by defects in the assembly of dolichol pyrophosphate oligosaccharide and/or in the transfer of oligosaccharide from dolichol pyrophosphate to an Asn residue on the nascent proteins. The other group, CDG II, reflects defects in the processing of protein-bound glycans. Only a few cases have been described *(1)*.

The diagnosis of CDG I is based on biochemical changes involving a unique carbohydrate deficiency observed in serum transferrin (TRF). In healthy subjects, serum TRF is fully glycosylated, containing two N-glycan chains, whereas in CDG I patients, it is partially (one chain) or totally deglycosylated *(3)*. This structural abnormality is associated with different enzyme deficiencies *(4)*. The most common, subtype Ia, is a deficiency of phosphomannomutase (PMM; EC 5.4.2.8) *(5)* and is present in 70% of CDG I patients. The disease is linked to chromosome 16p13, and numerous missense mutations have been identified in the *PMM2* gene *(6, 7)*. The condition is an autosomal recessive multisystemic disorder affecting the nervous system and numerous organs, including the liver, kidney, heart, adipose tissue, bone, and genitalia *(4)*.

The characteristic biochemical abnormalities of CDG can be demonstrated by various methods, including microanion-exchange chromatography or isoelectric focusing of TRF *(8)*, based on sialic acid content, and Western-blot analysis of plasma glycoproteins *(9)*, based on variations of protein molecular weight. Fig. 1A shows typical isoelectric focusing patterns for serum from a healthy subject and a CDG I patient; Fig. 1B shows typical Western-blot patterns for serum TRF, α_1-antitrypsin, haptoglobin, and α_1-acid glycoprotein from a healthy subject and a CDG I patient. The detection limit of the Western-blot method, tested by serial dilution, was <1 ng on the gel regardless of the glycoprotein tested. No discordance was observed between the TRF Western-blot assays and isoelectric focusing when >20 CDG I patient patterns were compared (data not shown).

We report here two cases of CDG Ia for which the condition could not be detected as easily as usual.

In the first family (F1), the sibling pair was composed of a 16-year-old girl (F1J) and a 6-year-old boy (F1D). Both have classical clinical features of CDG I, including psychomotor retardation, cerebellar ataxia, strabismus, and cerebellar hypoplasia; the girl also has hypogonadism. When Western blotting of the four different glycoproteins was performed on sera from both children, the results were puzzling. The boy's results showed a characteristic CDG I pattern (Fig. 1B, lane 4), consistent with the clinical features. By contrast, the pattern of serum glycoproteins of the girl (Fig. 1B, lane 3) was identical to the one of healthy subjects. The serum carbohydrate-deficient transferrin (CDT) was measured for both siblings (reference interval, 10–30 units/L CDT; F1J, 38 units/L CDT; F1D, 148 units/L CDT) and was consistent with the results of