Vitros Digoxin Immunoassay Evaluated for Interference by Digoxin-like Immunoreactive Factors, Barbara A. Way,1,2 Timothy R. Wilhite,3 Ralph Miller,4 Carl H. Smith,1,2 and Michael Landt1* (Departments of 1 Pediatrics and 2 Pathology, Washington University School of Medicine, St. Louis Children’s Hospital, and 3 BJC Health System, St. Louis, MO 63110; * author for correspondence: Department of Pediatrics, Washington University School of Medicine, St. Louis Children’s Hospital, One Children’s Place, St. Louis, MO 63110; fax 314-454-2274, e-mail landt@kids.wustl.edu)

Serum digoxin concentrations are routinely monitored because of the narrow therapeutic range of digoxin. Endogenous digoxin-like immunoreactive factors (DLIFs) may cause a false analytic increase in the measured digoxin concentration of patients undergoing digoxin treatment (1, 2). DLIF interference is most commonly found in the sera of neonates, and cord blood and neonatal sera frequently produce false positive results for digoxin (3, 4). Here we examine the newly introduced Vitros digoxin immunoassay (DGXN, Johnson & Johnson Clinical Diagnostics) performed on the Vitros 250 analyzer and compare it to a method (OnLine, Roche Diagnostic Systems) substantially free from DLIF interference (5) and to a method (Digoxin II, Abbott Laboratories) where DLIF interference is known to be present (1). These particular methods are not unique with regard to interference by DLIFs, and methods vary considerably in the impact of DLIF interferences (1, 2, 6, 7).

Serum samples used in this study were aliquots of samples that had been sent to St. Louis Children’s Hospital or Barnes-Jewish laboratories (BJC Health System) for the routine measurement of serum digoxin or aliquots of samples received for other routine chemistry analyses. Samples were from newborns (<2 weeks of age) and from adults. The study followed a protocol approved by the Human Studies Committee of Washington University. All samples were analyzed immediately or stored at −20 °C until use, with no more than three freeze-thaw cycles preceding analysis.

The Vitros DGXN digoxin assay is an enzymatic heterogeneous, competitive immunoassay that uses dry slide technology. Digoxin-peroxidase conjugate and sample digoxin compete for a limited number of binding sites on the immobilized antibody reagent. After the unbound conjugate is eluted and the leuco dye substrate is added, the rate of dye formation is inversely proportional to the concentration of digoxin in the sample. The OnLine digoxin assay (Roche) is a homogeneous microparticle immunoassay based on the aggregation of digoxin-coated microparticles in the presence of anti-digoxin antibody. Digoxin in the sample partially inhibits aggregation, and the rate of aggregation (as measured by light scattering) is inversely related to the digoxin concentration. The Digoxin II assay (Abbott) is a homogeneous, microparticle enzyme immunoassay that utilizes digoxin-alkaline phosphatase conjugate and the substrate 4-methylumbelliferyl phosphate. The concentration of the fluorescent final product of the reaction is inversely proportional to the concentration of digoxin in the sample.

Preliminary experiments determined the linearity of the new Vitros DGXN digoxin immunoassay and compared the recovery of digoxin by the new method to a method known to be free of DLIF interference (5) (Roche OnLine, performed on a COBAS FARA II analyzer). Linearity was determined by analysis of a serum pool (free of digoxin) to which digoxin in concentrations from 0 to 4 μg/L was added. Samples from this pool were split for analysis, and the linearity of the two assays was compared. The Roche OnLine and Vitros DGXN methods were linear throughout the range of clinical interest, but both methods exhibited a positive bias that was greater for the Vitros DGXN method (y = 1.28x + 0.00 μg/L) than the Roche method (y = 1.14x + 0.08 μg/L).

Between-run precision of the Vitros DGXN method was assessed by repeat analysis of two concentrations of quality control media (Bio-Rad levels 1 and 3, TDM Control, Bio-Rad). Coefficients of variation (n = 22) were 13.6% at 0.72 ± 0.10 μg/L and 3.5% at 2.88 ± 0.10 μg/L, respectively. The comparable values for the quality control media using the Roche OnLine method were 14.8% at 0.81 ± 0.12 μg/L and 4.7% at 3.20 ± 0.15 μg/L (n = 18 and 25, respectively). Addition of hemolysate to a serum containing 2.2 μg/L digoxin produced no interference in the Vitros DGXN method at concentrations of added hemolysate that yielded up to 10 g/L added hemoglobin.

We compared three digoxin assays using serum from 26 adults receiving digoxin therapy. Samples were split into three aliquots and then assayed for digoxin by all three methods (Fig. 1). The correlation of Vitros DGXN with Roche OnLine or Abbott Digoxin II was close (r = 0.99 and 0.98, respectively), and least-squares linear regression analysis computed close relationships of the Vitros method to the comparison methods [Vitros = 1.16(Abott) − 0.1 μg/L; S = 0.1 μg/L; Vitros = 0.97(Roche) + 0.2 μg/L, S = 0.1 μg/L]. The mean digoxin concentrations measured on the 26 comparison samples were 1.30 ± 0.69 μg/L (Roche OnLine), 1.34 ± 0.58 μg/L (Abbott Digoxin II), and 1.46 ± 0.68 μg/L (Vitros DGXN). These means were significantly different (Student’s t-test, paired), with P = 0.0001 for Vitros DGXN vs Roche OnLine and P = 0.009 for Vitros DGXN vs Abbott Digoxin II.

To investigate potential interference of DLIFs in the Vitros DGXN method, sera from 44 newborns (<30 days of age) not receiving digoxin therapy were split into three aliquots as above and measured by all three methods. Because directly quantifying the DLIF content of these specimens was not possible, we relied on the previously demonstrated high prevalence of DLIFs in neonatal specimens to provide a basis for comparison of DLIF interference in the three methods (3, 4). In 41 of 44 samples, the Vitros DGXN method gave measurements reported as <0.3 μg/L digoxin (instrument automatically reports <0.3 μg/L when a result below this threshold is obtained). The Roche OnLine method measured 0.0 ± 0.0 μg/L, and the Abbott Digoxin II method measured 0.5 ±
The potential sensitivity of the DGXN assay to interference from DLIFs was further assessed by adding digoxin to serum samples from both newborns (which probably contain DLIFs) and adults not under digoxin therapy (at 0.40 µg/L and 0.70 µg/L, respectively). This approach allowed for evaluation of bidirectional interference. The samples from newborns (n = 20) produced values of 0.41 ± 0.1 µg/L digoxin when assayed with the Roche OnLine method and 0.7 ± 0.2 µg/L when assayed with the Vitros DGXN digoxin method. The samples from adults (n = 6) produced values of 0.7 ± 0.0 µg/L digoxin when assayed with the Roche OnLine method and 0.8 ± 0.1 µg/L when assayed with the Vitros DGXN digoxin method.

The data taken as a whole suggest that the positive bias observed in the Vitros DGXN assay in comparison with the Roche OnLine assay of neonatal specimens is probably caused, in part, by the presence of DLIFs, which were to a slight degree detected as digoxin, and to a small analytical positive bias observed in comparisons of adult specimens and in recovery experiments. By subtracting the difference between the methods for adult specimens (0.13 µg/L) from the difference for neonatal specimens (0.24 µg/L), the net apparent digoxin in neonatal specimens (0.11 µg/L) in the Vitros DGXN digoxin method is probably caused by co-measurement of DLIFs.

The Vitros DGXN digoxin immunoassay was sufficiently sensitive and precise for clinical applications and was not influenced by hemolysis. The Vitros method produced results with a small overall positive bias compared with the Roche OnLine method, which has been reported to be free of DLIF interference (5). In 3 of 44 neonatal samples, there was a difference in the measurement of digoxin that could be attributed to the positive bias of the Vitros DGXN method plus the detection of some DLIFs. The extent of the differences (average difference, 0.24 µg/L; maximum difference, 0.5 µg/L) may influence clinical decision-making. We found no evidence, however, of the systematic detection of large amounts of DLIFs by the Vitros DGXN method. We conclude that DLIFs interfere slightly with the Vitros DGXN method, and the resulting small positive bias may cause occasional discrepant results.

References

Quantitative Determination of Short-Chain Organic Acids in Urine by Capillary Electrophoresis, Coral Barbas,* Natalia Adeva, Rosa Aguilar, Marta Rosillo, Teresa Rubio, and Mario Castro (Facultad de CC Experimentales y Técnicas, Universidad S. Pablo-CEU, Urbanización Montepríncipe Ctra. Boadilla del Monte, km 5,3-28668 Madrid, Spain; *author for correspondence: fax (91) 3510475, e-mail cbarbas@ceu.es)

Analysis of organic acids is a powerful technique in the diagnosis of inborn errors of metabolism characterized by organic aciduria, either by the excretion of excessive amounts of urinary organic acids ordinarily expressed or by the presence of organic acids rarely present in urine. The development of more reliable and reproducible analytical methods has shown that ill neonates and children who have primary disorders of organic acid metabolism are more frequent than previously thought (1), and early