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# A New Enzyme-Linked Chemiluminescent Immunosorbent Digoxin Assay Is Virtually Free From Interference of Spironolactone, Potassium Canrenoate, and Their Common Metabolite Canrenone

**Amitava Dasgupta,[1]* Edward Kang,[2] and Pradip Datta[2]**

[1]*Department of Pathology and Laboratory Medicine, University of Texas-Houston Medical School, Houston, Texas*

[2]*Bayer HealthCare, Diagnostics Division, Tarrytown, New York*

Spironolactone and potassium canrenoate (aldosterone antagonist diuretics) are sometimes used in conjunction with digoxin for patient management. Spironolactone, potassium canrenoate, and their common metabolite canrenone interfere with serum digoxin measurement using various immunoassays. Recently a new enzyme-linked chemiluminescent immunosorbent digoxin assay (ECLIA-Digoxin) became commercially available for application on the ADVIA IMS 800i modular system (Bayer HealthCare, Tarrytown, NY). We investigated the potential interference of spironolactone and related compounds in this assay by comparing the results with the fluorescence polarization immunoassay (FPIA), which is known to have significant cross-reactivity with these compounds as well as a turbidimetric assay for digoxin with no known cross-reactivity with spironolactone and related compounds. Aliquots of drug free serum were supplemented with therapeutic and above therapeutic concentrations of spironolactone, canrenone, and potassium canrenoate, and apparent digoxin concentrations were measured. No apparent digoxin concentration was observed using the ECLIA-Digoxin or turbidimetric assay. When serum pools prepared from patients receiving digoxin were further supplemented with these compounds, we observed no significant change in digoxin concentrations in the presence of these compounds with the ECLIA-Digoxin. We conclude that this assay is virtually free from interferences from spironolactone, potassium canrenoate and their common metabolite canrenone. J. Clin. Lab. Anal. 20:204–208, 2006.


**Key words:** spironolactone; metabolite; interference; fluorescence polarization immunoassay; turbidimetric assay; enzyme-linked immunosorbent assay

## INTRODUCTION

Digoxin is a cardiac glycoside prescribed to increase the adequacy of circulation in patients with congestive heart failure and also to slow ventricular rate in the presence of atrial fibrillation and flutter. Spironolactone, a competitive aldosterone antagonist, has been used clinically in the therapy of hypertension and congestive heart failure. Spironolactone is metabolized to canrenone, an active metabolite. Although not used in the US (1), potassium canrenoate is used in Europe and other parts of the world. Potassium canrenoate, like spironolactone, is also metabolized to canrenone (2,3). Potassium canrenoate, spironolactone, and canrenone all have structural similarity with digoxin resulting in interference with various digoxin immunoassays.

Digoxin has a narrow therapeutic range (0.8–1.9 ng/mL), and toxicity may be encountered in a digoxin concentration as low as 2.0 ng/mL. Morris et al. (4) reported positive interference of spironolactone in digoxin measurement using the fluorescence polarization immunoassay (FPIA) in 1988. Later, other authors also confirmed the interference of spironolactone and canrenone in the FPIA and other commonly used

*Correspondence to: Dr. Amitava Dasgupta, Department of Pathology and Laboratory Medicine, University of Texas-Houston Medical School, 6431 Fannin, MSB 2.292, Houston, TX 77030. E-mail: Amitava.Dasgupta@uth.tmc.edu

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immunoassays for digoxin (5–7). Okazaki et al. (8) reported two cases where cross-reactivity of the FPIA assay caused clinical problems and therefore recommended the use of OPUS digoxin assay, which showed minimum cross-reactivity.

Canrenone demonstrated significant negative interference with the microparticle enzyme immunoassay (MEIA) of digoxin on the AxSYM analyzer (Abbott, Abbott Park, IL) leading to a falsely low digoxin reading. Misleading sub-therapeutic concentrations of digoxin, as measured on several occasions, led to incorrect digoxin dosing and digoxin toxicity in patients (9,10). Suppression of digoxin values due to the presence of canrenone was also observed using the Dimension (run on the acaSX analyzer, Dade Behring) digoxin assays. In contrast, a positive bias (falsely elevated digoxin values) was observed with the aca (run on the acaSX analyzer, Dade Behring, Deerfield, IL), FPIA (run on the TDx analyzer, Abbott Laboratories, Abbott Park, IL), and Elecsys (run on the Elecsys 2000 analyzer, Roche, Indianapolis, IN) digoxin assays. Strongest positive bias was caused by canrenone, the common metabolite of spironolactone and potassium canrenoate. The EMIT 2000 digoxin immunoassay (Dade Behring), Tina-Quant digoxin assay (Roche), and Vitros digoxin assay (Ortho Clinical Diagnostics, Rochester, NY) did not demonstrate any significant interference (10).

Although high dose spironolactone therapy may cause falsely lower digoxin values as measured by the MEIA assay, low dose spironolactone therapy (up to 25 mg/day) may not affect digoxin concentrations as measured by the MEIA assay on the AxSYM analyzer (11). We reported earlier that the turbidimetric digoxin immunoassay (TIA) on the ADVIA 1650 analyzer (Bayer Health Care) is free from interference by spironolactone, potassium canrenoate, and their common metabolite canrenone (12). Recently Bayer HealthCare, Diagnostics Division released a new enzyme-linked chemiluminescent immunosorbent digoxin assay (ECLIA-Digoxin) for application on the ADVIA IMS 800i modular system (Bayer HealthCare, Tarrytown, NY). Potential interference of spironolactone, potassium canrenoate, and their common metabolite canrenone on this digoxin assay has not been studied before. Here we report our findings on potential interference of these compounds using the ECLIA-Digoxin assay.

## MATERIALS AND METHODS

Spironolactone and canrenoic acid potassium salt (potassium canrenoate) were purchased from the Sigma Chemical Company (St. Louis, MO). We prepared canrenone by acid-catalyzed lactonization of potassium canrenoate according to Tal (13) except that we used p-toluenesulfonic acid as a catalyst. Canrenone was purified by crystallization using ethyl acetate as described by Tal (13). The FPIA kits for digoxin were purchased from the Abbott Laboratories. The FPIA assay was run on an FLx/TDx analyzer (Abbott Laboratories). The ECLIA-Digoxin assay was run on the ADVIA IMS 800i modular system while the TIA was run on the ADVIA 1650 analyzer (Bayer HealthCare).

The FPIA digoxin assay utilizes fluorescence polarization immunoassay technology and uses a polyclonal rabbit digoxin antiserum. The assay requires sample pretreatment prior to analysis and is linear up to a serum digoxin concentration of 5.0 ng/mL. The detection limit of the assay is 0.20 ng/mL. The ECLIA-Digoxin assay is a modification of the enzyme-linked immunosorbent technique. In this homogenous competitive immunoassay, digoxin antibody conjugate (fluorescein isothiocyanate (FITC), a fluorescent tag) reacts (R1) with the specimen at 37°C. Then a digoxin enzyme (alkaline phosphatase) conjugate (R2) is added. FITC-antibody-digoxin-ALP (ALP = alkaline phosphatase) is the final product, which is water soluble, and is now pulled out by magnetic particles conjugated to anti-FITC antibody. After the bound enzyme is washed, the substrate (3-(2′spiroadamantane)-4-methoxy-4-(3′-phosphorylaxy)phenyl-1,2-dioxetane, AMPPD) is added and the chemiluminescent is measured for the signal. This assay has a detection limit of 0.04 ng/mL of digoxin. The TIA is a latex-enhanced immunoturbidmetric assay that is run on the continuous, random access ADVIA 1650 system. The assay is linear up to a serum digoxin concentration of 5.0 ng/mL, and the detection limit of the assay is 0.1 ng/mL. Even though both TIA and ECLIA digoxin assays use monoclonal mouse digoxin antibodies, the antibodies are of different clones and the assays have different design.

Aliquots of drug free serum were supplemented with various concentrations of spironolactone, canrenone, or potassium canrenoate, and apparent digoxin concentrations were measured by FPIA turbidimetric and ECLIA-Digoxin assays. For the next experiment, two serum pools were prepared using sera from patients receiving digoxin. We routinely receive serum for therapeutic monitoring of digoxin in our clinical laboratory. After performing and reporting all results to the ordering clinician, we discard these specimens after one week. For this study, we used leftover specimens after removing the identity of the patients. These specimens would otherwise be discarded. Then aliquots of these digoxin pools were supplemented with various amounts of spironolactone, canrenone, and/or potassium canrenoate. Digoxin concentrations were measured using all three digoxin immunoassays in

triplicate, and values were expressed as the mean and one standard deviation.

In a different experiment, cross-reactivity of various digoxin metabolites with ECLIA-Digoxin assay was studied by supplementing various digoxin pools (prepared by adding digoxin to drug free serum) with different amounts of digoxin metabolites. The cross-reactivity of different metabolites was calculated by comparing digoxin values before and after supplementation of digoxin metabolites.

Statistical analysis was done using independent t-test, two tailed. We considered a difference to be significant only at 95% confidence interval or higher.

## RESULTS

We considered an apparent digoxin concentration as "none detected" if the observed value was lower than the detection limit of the individual digoxin immunoassay. We observed no apparent digoxin concentration when aliquots of drug free serum pool were supplemented with various amounts of spironolactone, potassium canrenoate, and canrenone. Digoxin concentrations were measured by the new ECLIA-Digoxin assay. As expected no apparent digoxin concentration was observed using the turbidimetric digoxin assay, but significant apparent digoxin concentrations were observed using the FPIA assay (Table 1).

Rigorous characterization of interference in an immunoassay due to a cross reactant should be performed in the presence of the primary analyte (14). Therefore, we added spironolactone, potassium canrenoate, and canrenone, the cross reactants in digoxin pools prepared from patients receiving digoxin, and then we measured apparent digoxin concentrations for comparison with the original digoxin concentrations. The concentrations of spironolactone, potassium canrenoate, and canrenone selected for supplementation were based on published reports (15–17) of concentrations of these drugs or metabolites found in serum after therapy with spironolactone or potassium canrenoate. We observed significant increases in apparent digoxin concentrations in the presence of these cross reactants using the FPIA digoxin assay only. In contrast, no significant difference was observed in digoxin concentration in the presence of spironolactone, potassium canrenoate, and canrenone in most specimens when ECLIA-Digoxin assay was used indicating that this assay is virtually free from interference of these compounds. With digoxin pool 2, in the presence of 1,000 ng/mL of canrenone, the observed digoxin concentration of 1.06 ng/mL (SD = 0.02) as observed with ECLIA-Digoxin assay was significantly greater than the observed original digoxin concentration of 0.99 ng/mL (SD = 0.03). However, this 7.0% increase in digoxin concentration is clinically insignificant. Moreover, this value may be due to a random error because the observed digoxin concentration in the presence of a much higher canrenone concentration of 2,000 ng/mL had a digoxin value of 0.98 ng/mL (Table 2).

Digoxin metabolites; digoxygenin bis-digitoxide, and digoxygenin mono-digitoxoside demonstrated significant cross-reactivity with ECLIA-Digoxin assay while the cross-reactivity of dihydrodigoxin was negligible (Table 3).

**TABLE 1. Cross-reactivity of spironolactone, potassium canrenoate, and canrenone with FPIA, turbidimetric, and new ECLIA-digoxin assays**

| | Apparent Digoxin (ng/mL, n = 3) [mean (SD)] | | |
|---|---|---|---|
| Specimen | FPIA | Turbidimetric | ECLIA-digoxin |
| Drug/DLIS free serum+spironolactone | None detected | None detected | None detected |
| 100 ng/mL | None detected | None detected | None detected |
| 250 ng/mL | None detected | None detected | None detected |
| 500 ng/mL | 0.21 (0.01) | None detected | None detected |
| 1,000 ng/mL | 0.26 (0.02) | None detected | None detected |
| Drug/DLIS free serum+potassium canrenoate | | | |
| 250 ng/mL | 0.28 (0.04) | None detected | None detected |
| 500 ng/mL | 0.33 (0.07) | None detected | None detected |
| 1,000 ng/mL | 0.38 (0.08) | None detected | None detected |
| 2,000 ng/mL | 0.54 (0.03) | None detected | None detected |
| Drug/DLIS free serum+canrenone | | | |
| 250 ng/mL | 0.27 (0.04) | None detected | None detected |
| 500 ng/mL | 0.35 (0.08) | None detected | None detected |
| 1,000 ng/mL | 0.57 (0.06) | None detected | None detected |
| 2,000 ng/mL | 0.76 (0.05) | None detected | None detected |

**TABLE 2. Effect of spironolactone, potassium canrenoate, and canrenone on serum digoxin concentrations as measured by FPIA, turbidimetric, and ECLIA digoxin assays**

| | Digoxin concentration (ng/mL, n = 3) [mean (SD)] | | |
|---|---|---|---|
| Specimen | FPIA | Turbidimetric | ECLIA-digoxin |
| Digoxin pool 1 | 1.44 (0.05) | 1.51 (0.06) | 1.63 (0.04) |
| +500 ng/mL spironolactone | 1.49 (0.04) | 1.48 (0.02) | 1.60 (0.02) |
| +1,000 ng/mL spironolactone | 1.55 (0.06)[a] | 1.46 (0.02) | 1.63 (0.03) |
| +1,000 ng/mL K-canrenoate | 1.68 (0.02)[a] | 1.51 (0.12) | 1.63 (0.02) |
| +2,000 ng/mL K-canrenoate | 1.90 (0.07)[a] | 1.53 (0.02) | 1.64 (0.01) |
| +1,000 ng/mL canrenone | 1.76 (0.10)[a] | 1.51 (0.08) | 1.57 (0.02) |
| +2,000 ng/mL canrenone | 1.97 (0.05)[a] | 1.53 (0.02) | 1.55 (0.01)[b] |
| +1,000 ng/mL S +1,000 ng/mL canrenone | 1.86 (0.03)[a] | 1.49 (0.04) | 1.57 (0.02) |
| +1,000 ng/mL K-C +1,000 ng/mL canrenone | 2.03 (0.03)[a] | 1.49 (0.06) | 1.56 (0.02) |
| +1,000 ng/mL K-C +2,000 ng/mL canrenone | 2.24 (0.05)[a] | 1.48 (0.06) | 1.55 (0.04) |
| Digoxin pool 2 | 0.91 (0.04) | 0.92 (0.06) | 0.99 (0.03) |
| +500 ng/mL Spironolactone | 0.96 (0.02) | 0.93 (0.02) | 1.03 (0.02) |
| +1,000 ng/mL Spironolactone | 1.03 (0.06)[a] | 0.90 (0.04) | 1.03 (0.02) |
| +1,000 ng/mL K-canrenoate | 1.14 (0.06)[a] | 0.94 (0.03) | 1.04 (0.03) |
| +2,000 ng/mL K-canrenoate | 1.26 (0.03)[a] | 0.91 (0.05) | 1.08 (0.05) |
| +1,000 ng/mL canrenone | 1.18 (0.04)[a] | 0.90 (0.02) | 1.06 (0.02)[a] |
| +2,000 ng/mL canrenone | 1.29 (0.07)[a] | 1.02 (0.12) | 0.98 (0.01) |
| +1,000 ng/mL S +1,000 ng/mL canrenone | 1.19 (006)[a] | 0.92 (0.01) | 0.99 (0.02) |
| +1,000 ng/mL K-C +1,000 ng/mL canrenone | 1.38 (0.03)[a] | 0.98 (0.08) | 0.99 (0.01) |
| +1,000 ng/mL K-C +2,000 ng/mL canrenone | 1.52 (0.09)[a] | 0.88 (0.06) | 0.96 (0.03) |

[a]Significantly greater than the control value by independent *t*-test, two-tailed ($P<0.05$).
[b]Significantly less than the control value by independent *t*-test, two-tailed ($P<0.05$).
K-C, potassium canrenoate.

**TABLE 3. Effect of supplementing digoxin pools with digoxin metabolites on serum digoxin measurements by the ECLIA-digoxin assay**

| | | Digoxin (ng/mL) | | |
|---|---|---|---|---|
| Metabolite | Amount added (ng/mL) | Prespike | Postspike | Cross-reactivity |
| Digoxygenin | 40 | 0.75 | 3.33 | 6.4% |
| | 40 | 1.90 | 4.18 | 5.7% |
| | 40 | 3.42 | 5.52 | 5.2% |
| Digoxygenin bis-digitoxoside | 1.5 | 0.42 | 1.12 | 46.6% |
| | 1.5 | 1.67 | 2.21 | 36.0% |
| | 1.5 | 2.73 | 3.42 | 46.0% |
| Digoxygenin mono-digitoxoside | 0.8 | 0.42 | 0.94 | 65.4% |
| | 0.8 | 1.67 | 2.09 | 52.5% |
| | 0.8 | 2.73 | 3.27 | 67.5% |
| Dihydrodigoxin | 120 | 0.77 | 0.86 | 0.07% |
| | 120 | 1.90 | 1.99 | 0.08% |
| | 120 | 3.36 | 3.37 | 0.01% |

## DISCUSSION

Oral administration of 100 mg spironolactone (moderate dose) usually leads to a peak serum spironolactone concentration of 83 ng/mL and peak canrenone concentration of 202 ng/mL (15). After intravenous administration of potassium canrenoate, the peak plasma

canrenone concentration was 2,066 ng/mL. However, the peak canrenone concentration could also be as low as 1,117 ng/mL (16). Sadee et al. (17) studied pharmacokinetics of spironolactone, canrenone, and potassium canrenoate in humans. The authors used either an oral dose of 400 mg of spironolactone or an intravenous dose of 380 mg of potassium canrenoate. Although initial plasma concentration of potassium canrenoate was high, the value rapidly dropped to 1,000 ng/mL after achieving steady state. The mean plasma concentration of canrenone was 1,400 ng/mL (17). The in vitro concentrations we selected for this study include expected concentrations of spironolactone, potassium canrenoate, and their common metabolite canrenone after a low to high dose of both spironolactone and potassium canrenoate therapy as well as an overdose of both.

Interference of spironolactone and related drugs in the therapeutic drug monitoring of digoxin using immunoassay may be troublesome. Morris et al. (18) studied eight commercial digoxin immunoassays in 17 subjects taking spironolactone, but not digoxin, to evaluate potential interference of spironolactone and the metabolite on digoxin immunoassays. The authors reported that four out of eight immunoassays studied showed significant cross-reactivity. Steimer et al. (10) commented that negative interference in digoxin immunoassay (MEIA, Abbot Laboratories), due to spironolactone and related compounds, is much more dangerous than the positive interference as observed with some digoxin immunoassays. Therefore, a digoxin immunoassay that is virtually free from the interference of spironolactone and related compounds is most suitable for therapeutic drug monitoring of digoxin in patients receiving spironolactone or potassium canrenoate.

Our results indicate that the new ECLIA-digoxin assay is virtually free from the interference of spironolactone, potassium canrenoate, and their common metabolite canrenone. This may be related to the specificity of monoclonal antibody against digoxin that is used in this assay. We reported earlier that this assay is also free from interference of endogenous digoxin-like immunoreactive substances (DLIS) (19). It is interesting that digoxin metabolites retaining one or two sugars showed significant cross-reactivity with ECLIA-Digoxin assay but digoxygenin with no sugar moiety showed very little cross-reactivity with this assay. Lack of cross-reactivity of spironolactone, potassium canrenoate, and canrenone with this assay may be due to lack of a sugar molecule in these compounds. We conclude that the new ECLIA-Digoxin assay is virtually free from interference of spironolactone and related compounds and is suitable for therapeutic drug monitoring of digoxin in patients also receiving spironolactone or potassium canrenoate.

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