# A rapid easy-to-perform solid phase digoxin radioimmunoassay

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**Abstract** A solid-phase-radioimmunoassay (SPRIA) for the monitoring of blood digoxin level has been developed, in which a secondary antibody-coated polystyrene tubes are used. This noval method seems to be simple to use and only takes about an half hour. The standard curve is linear from 0.25 to 4 $\mu$g/L. The sensitivity of the detection is 0.1 $\mu$g/L. Reproducibility studies with 3 control sera of 0.5~2.5 $\mu$g/L give intraassay CV< 5% and interassay CV< 10%. The specimens are measured and compared with those of the conventional radioimmunoassay and the values are well correlated ($r$=0.96, $Y$=1.022$X$+0.04 $\mu$g/L).
**Keywords** SPRIA, Digoxin, Serium

## 1 Introduction

The standard RIA-type assay suffers inherent disadvantages, such as too many steps, too much operation time and expensive instruments used. The double monoclonal sandwich-type assay requires several different antibodies in large quantity, and these antibodies used are also expensive. Therefore, SPRIA based on second antibody-coated polystyrene tubes, which overcomes the disadvantages of two methods mentioned above, has been developed.

## 2 Materials and methods

### 2.1 Materials

60×12 mm polystyrene tubes were used as solid matrices; primary anti-digoxin serum raised in rabbits was preserved in 0.1 ml alquots at -20°C; sheep anti-rabbit secondary antiserum was from SIGMA; agent digoxin was commercially available; $Na^{125}I$, about 629 MBq/$\mu$g, was from Amersham, U.K; quality control sera were from Drug Administration of China and preserved in 0.5 ml aliquots at -20°C.

### 2.2 Iodination of digoxin

Radioiodinated digoxin was prepared as described in Refs.[1,2] and purified through Sephadex-A25.

### 2.3 Antibody-coated solid tubes

Polystyrene tubes containing 0.6 ml of secondary antiserum in 0.01 mol/L sodium phosphate buffer (pH7.6) were incubated for 24 h at room temperature; then washed with buffer containing 1% bovine serum albumin for 4 h; finally, dried in air and stored at 4°C in sealed bags and ready for use.

### 2.4 SPRIA and RIA methods

In SPRIA, 25$\mu$l of serum sample (or control serum, standard), 100$\mu$l primary antibody (1:20000 dilution) and 200$\mu$l of $^{125}$I-digoxin were pipetted into the bottom of the antibody coated-tube. It was incubated with mild shaking (200 r/min) at 37°C for 20 min. After the content in each tube was aspirated carefully, the tubes were washed with 1 ml of washing solution. The radioactivity of the tubes was then measured immediately. Finally, the results were found based on the standard curve.

In RIA, 25$\mu$l of serum sample (or control serum, standard), 200$\mu$l of $^{125}$I-digoxin and 100$\mu$l of primary anti-digoxin antibody (1:5000) were added into the bottom of the uncoated tube, and incubated for 20 min at 37°C. Then, PEG was used to precipitate the primary antibody-digoxin complex for 15 min at 4°C. The supernatant was carefully removed, and the radioactivities of the tubes were measured.

### 2.5 Correlation of SPRIA with RIA

Side by side determinations of digoxin were performed with both methods on 20 specimens (freshly thawed serum, 0.2~ 2.8$\mu$g/L) and the correlation was determined by regression analysis; a replicate analysis of the zero calibrator was used to estimate sensitivity of SPRIA; precision was studied by the analysis of quality

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controls at three concentrations; 10 replicate assays were used to establish the intra-assay variation of SPRIA, and 5 consecutive runs provide the inter-assay variation; linearity of SPRIA response to digoxin concentration was investigated by analyzing serial dilutions of patients' specimens; the added amounts as well as the endogenous amounts of digoxin were determined by SPRIA for recovery analysis.

## 3 Results

Standard curve for SPRIA is shown in Fig.1. The correlation of SPRIA with RIA is shown in Fig.2, the regression equation $Y = 1.022X+0.04\mu g/L$ ($r$=0.96).

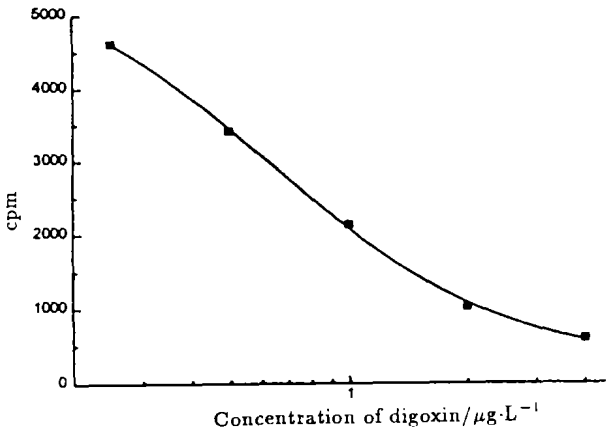


**Fig.1** Standard curve for SPRIA of digoxin

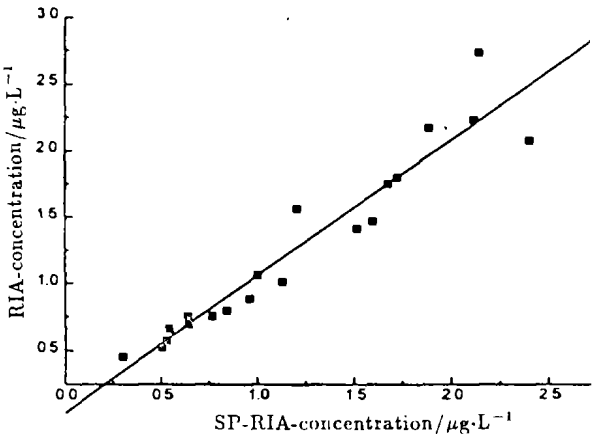


**Fig.2** Correlation between SPRIA and RIA $n$=20

The sensitivities of SPRIA and RIA were 0.1$\mu$g/L and 0.2$\mu$g/L, respectively. Reproducibility data from the three control sera series are summarized in Table 1.

**Table 1** Precision of SPRIA assay

| Digoxin/$\mu g \cdot L^{-1}$ | Intra-assay CV/% | Digoxin/$\mu g \cdot L^{-1}$ | Interassay CV/% |
|---|---|---|---|
| 0.87 | 2.58 | 0.62 | 9.7 |
| 1.40 | 4.96 | 1.21 | 6.7 |
| 2.10 | 3.58 | 1.77 | 7.8 |

The SPRIA assay curve was linear within 0.25~4$\mu$g/L. Regression equation was $y = 0.96x$-0.046$\mu$g/L with $r$=0.999 between expected and observed values. Recovery rate averaged 99.9%

## 4 Discussion

Digoxin is one of the most important drugs for the treatment of congested heart failure. In many instances, serum concentration of digoxin both within and among individuals cannot be accurately predicted from the recommended dose of drug.[3] The narrow effective therapeutic plasma concentration ranging from 0.8~ 2.0$\mu$g/L has been established for accurate assessement of the serum concentration of digoxin.[4] The preponderance of the laboratory test have relied on digoxin values obtained from two assay, i.e. the double sandwich-type assay and the standard RIA assay. Although, these assays have been well accepted by the medical community, but the cost of time and money are our major consideration for developing SPRIA method.

Our results using secondary antibody-coated polystyrene tube, provides substantial improvement of available digoxin assays. It of-

fers good reproducibility, linearity, sensitivity and reliability. In addition, there is an exceptional equivalence in digoxin values between the SPRIA assay and the standard RIA assay, however, the consumption of primary antibody by this SPRIA method is much less than that of RIA method, and the SPRIA is more convenient for use in clinical application. In conclusion, a rapid (30 min), easy-to-perform and sensitive assay capable of measuring serum digoxin has been developed.

## References

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