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CHARLES L. KROLL, Sc.D., director
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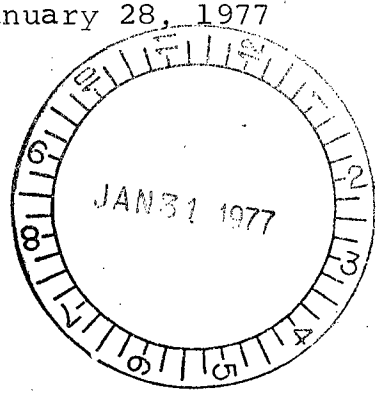
January 28, 1977




Nuclear Regulatory Commission
Washington, D. C. 20555

Attention: Mr. Nathan Bassin
Radioisotopes Licensing Branch

Gentlemen:

We hereby wish to supplement our specific license 29-00139-05G, which provides for the distribution of Iodine-125 containing in vitro products to persons generally licensed pursuant to 10 CFR § 31.11, to provide for an additional product, Digoxin CLASP (TM) RIA Kit; each prepackaged unit does not exceed 10 microcuries of Iodine-125.

Copies of the printed labeling components which contain the information required under § 32.71 (c)(1) and (2), (d), and (e) are enclosed. For the purposes of this supplemental application, only those portions of the brochure, which accompanies the package, containing information pertinent to § 32.71 (d) and (e) are being submitted for review; these are outlined in the attached brochure.

We trust that this information will be adequate and look forward to your approving this supplement.

Sincerely,

C. L. Kroll

/lc
Enclosure



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Revised _____

# Digoxin CLASP(TM) RIA Kit

## For Quantitative Measurement of Serum or Plasma Digoxin Levels by Radioimmunoassay

## For *IN VITRO* Diagnostic Use
## For Professional Use Only

**DETERMINATION OF SERUM OR PLASMA DIGOXIN LEVELS BY RADIOIMMUNOASSAY**

Measurement of body constituents or administered compounds by the technique of radioimmunoassay offers a bioanalytical tool that combines the extreme sensitivity of radioisotope methodology with the extreme specificity of immunological techniques. The procedure requires a specific antibody, a radiolabeled antigen, a pure sample of the antigen to serve as a reference standard, and a means of separation of free antigen from antibody-bound antigen. The procedure follows the basic principle of saturation analysis, where there is competition between labeled and unlabeled antigen for a fixed number of antibody binding sites. As the concentration of unlabeled antigen (the substance actually being measured) increases, less of the added radiolabeled antigen will be bound to the antibody. When equilibrium has been reached in the antigen-antibody reaction, the free and bound components of the mixture are separated, and the relative amounts of each are determined by measuring the radioactivity of the separated components. The absolute quantity of unlabeled antigen in the sample being analyzed is determined by comparing the assay results to a standard curve prepared with known amounts of the unlabeled antigen.

In the Digoxin CLASP RIA Kit, antibody to digoxin (raised in New Zealand white rabbits by administration of digoxin coupled to human serum albumin) serves as the specific antibody, purified digoxigenin labeled with $^{125}I$ serves as the labeled antigen, and purified digoxin is used as a reference standard. The specific antibody to digoxin is linked to the assay test tube to facilitate separation of free digoxin from antibody-bound digoxin. In this solid-phase system, the antibody-bound digoxin will remain on the walls of the test tube, and the free digoxin will be removed, when the incubate is poured from the test tube. Digoxin levels are expressed as nanograms (ng, $10^{-9}$ g) of digoxin per milliliter of serum or plasma (ng/ml).

## RATIONALE FOR USE

The measurement of serum or plasma levels of digoxin by radioimmunoassay has proved to be a valuable adjunct in the clinical diagnosis of digoxin toxicity. Since excessive accumulation of digoxin is a major factor in the development of toxicity, and there is some constancy in myocardium-to-serum digoxin ratios,[1] determination of serum digoxin concentration can be of help in the diagnosis of digoxin intoxication.[2]

Measurement of serum or plasma digoxin by radioimmunoassay has the advantages of speed and simplicity over the originally used double-isotope derivative method[3] and has the advantages of simplicity and specificity over bioassay methods.[4] The $^{86}Rb$ uptake method has yielded results that are in good agreement with results obtained by radioimmunoassay, but requires a day to obtain results versus one hour required for radioimmunoassay.[5]

The utilization of solid-phase technology greatly simplifies the separation procedure and reduces the manipulations associated with the test procedure.

The Digoxin CLASP RIA Kit utilizes $^{125}I$ labeled digoxigenin in lieu of $^{3}H$ labeled digoxin that is employed in many of the procedures described in the literature. The use of $^{125}I$ rather than $^{3}H$ avoids the problems associated with sample preparation and availability of liquid scintillation counting equipment. In addition, the use of internal counting standards to correct for quenching associated with the presence of variable quantities of bile pigments or hemoglobin[6] is avoided with the $^{125}I$ label. The half-life of $^{125}I$ is 60.2 days. The isotope decays in a complex fashion with emission of x-rays and gamma rays whose radiation energies are 27.4 keV and 35.4 keV, respectively. These energies are well within the detection capability of modern solid crystal gamma scintillation detectors. There is no beta emission.

## DIGOXIN CHEMICAL AND BIOLOGICAL PROPERTIES

***Chemical Properties:*** Digoxin is a pure glycoside obtained from the leaves of *Digitalis lanata*. Like all cardiac glycosides, digoxin consists of a steroidal portion or aglycone, and a glycosidic portion, consisting of three digitoxose sugar residues. Digoxin is formed upon partial hydrolysis of the naturally occurring Lanatoside C found in *Digitalis lanata*. It differs from digitoxin by the presence of an extra hydroxyl group at the C-12 position. Because of this structural difference, digoxin shows increased polarity and decreased lipid solubility, resulting in a marked difference in the pharmacokinetics of the two compounds.

The pharmacologic activity of cardiac glycosides is contained exclusively in the steroidal (aglycone or genin) portion of the molecule. The sugars possess no intrinsic activity, but they enhance the pharmacologic activity of the aglycone several times, presumably by increasing solubility or enhancing the ability of the drug to penetrate cell membranes. The pharmacologically active aglycone portion of the digoxin molecule, devoid of the sugar residues, is referred to as digoxigenin, and is the radiolabeled component of the Digoxin CLASP RIA Kit.

***Biological Properties:*** Digoxin is well absorbed from the gastrointestinal tract, with approximately 80 percent of an oral dose being eventually absorbed. Following oral administration, peak serum levels are found at one to two hours. Absorption is not diminished by food or fasting, although the shape of the curve defined by serum levels has a somewhat lower and more extended peak.[7]

Doherty[8] and co-workers administered tritium labeled digoxin to human subjects and found a more or less constant relationship between tissue and serum levels. Concentrations of digoxin were always highest in the heart, followed by liver and kidney. The ratio between heart and serum concentration was rather constant at 30:1.[8] The relationship between tissue and serum concentration and the fairly uniform serum concentration over several hours during the post-absorptive phase, provides the basis for the clinical use of serum digoxin determinations.[9]

There is some controversy as to the nature of the binding (specific vs. nonspecific), but it is generally accepted that digoxin forms a complex with the plasma membrane-bound enzyme, $Na^+$, $K^+$ ATP-ase. It has been shown that all cardiac glycosides inhibit ATP-ase activity in cardiac and other tissues, and that concentrations causing inhibition are in a range known to cause a positive inotropic effect.[7] As with other cardiac glycosides, digoxin acts primarily on the heart to (1) increase the force of systolic contraction; (2) slow conduction and lengthen the refractory period through the AV node and bundle of His; and (3) alter cardiac vagal activity.

Digoxin is excreted largely unchanged in the urine. Loss in the stool accounts for about 15 percent of a single dose, with virtually all of this being derived from bile. Of clinical importance is the direct relationship between glomerular filtration rate and the clearance of digoxin. Patients with renal impairment have a significantly prolonged serum half-life and tend to accumulate the drug. Anephric subjects exhibit a very long serum half-life and have markedly increased excretion via the stool, apparently representing a secondary adaptive mechanism for limiting the progressive accumulation of digoxin in such patients.[7]

## CLINICAL APPLICATIONS

The clinical use of digitalis and its component glycosides is accompanied by a distressingly high prevalence of toxic manifestations, the most serious of which are arrhythmias and disturbance of conduction. Many factors contribute to the development of digitalis toxicity, with perhaps the most important factor being an accumulation of excessive amounts of digitalis in the body and in the myocardium in particular.[10] Since there is a degree of correlation between serum and tissue levels of digoxin, the measurement of serum digoxin levels provides useful information in the diagnosis of digoxin toxicity. In view of the multiple factors governing individual response to cardiac glycosides, however, it must be stressed that serum digoxin measurements should be viewed as just one of many important factors to be weighed in a complex clinical setting.[11]

The measurement of serum digoxin levels, in conjunction with careful investigation of adherence to prescribed medication schedules, has also been of value in detecting patients who fail to comply with the prescribed dosage regimen.[12,13]

## CLINICAL BACKGROUND

Studies from a number of laboratories, using several different techniques, reflect substantial agreement concerning serum or plasma digoxin levels in patients receiving usual doses of this drug. Beller et al [14] conducted a prospective study of 931 consecutive patients admitted to a medical service at their institution, to describe the prevalence and epidemiology of cardiac digitalis toxicity, and to correlate serum concentrations of digoxin (measured by radioimmunoassay) with clinical and biochemical data. Fifteen percent of the patients surveyed were taking digitalis on admission, and of these, 23 percent were definitely toxic, and 6 percent possibly toxic, as determined by serial electrocardiograms. There was a significantly greater prevalence of advanced heart disease, underlying atrial fibrillation, anorexia, acute or chronic pulmonary disease, and renal failure in toxic patients, versus nontoxic patients. Mortality was more than twice as high in the toxic group versus the nontoxic group. The mean serum digoxin concentration in toxic patients was 2.3 ± 1.6 (±S D) ng /ml , whereas the mean level in the nontoxic group was 1.0 ± 0.5 ng /ml ($p<0.005$). The degree of overlap between the two groups was somewhat greater than reported by other investigators, and was attributed to the prospective nature of the study and the long time interval (i.e., up to 48 hours) between the last dose of digitalis and the performance of the serum digoxin assay. It was emphasized that because of the overlap of serum digitalis levels in clinically toxic and nontoxic patients, sole reliance on these levels for the determination of the presence or absence of digitalis toxicity is not warranted. Knowledge of serum glycoside levels was found most useful when weighed in the entire clinical context.

In a previous study, Smith and Haber[11] reported a mean serum digoxin level of 3.7 ± 1.0 (S D ) ng /ml for toxic patients, and a mean of 1.4 ± 0.7 ng /ml for nontoxic patients. Ninety percent of patients without evidence of toxicity had serum digoxin concentrations of 2.0 ng /ml or less, while 87 percent of the toxic group had levels above 2.0 ng /ml. Blood samples from these patients were obtained between 8 and 12 hours after the last dose of digoxin.

Johnston et al [15] reported mean plasma digoxin levels of 0.92 ± 0.19 ng /ml for adequately digitalized patients taking 0.25 mg of digoxin per day, while patients taking 0.5 mg per day had a mean plasma level of 1.23 ± 0.1 ng /ml. Twenty-one patients who fulfilled the criteria for digitalis toxicity had mean digoxin levels of 3.15 ± 0.25 ng /ml for the 0.25 mg / day dosage, and 3.10 ± 1.18 ng /ml for the 0.5 mg /day dosage. All but three of the toxic patients showed some degree of renal insufficiency. In this study, the blood was drawn for assay three hours after the last digitalis dose.

Zeegers et al [16] recently reported on a group of 93 well digitalized patients and 22 patients considered toxic. The mean digoxin level for the nontoxic patients was 1.6 ± 0.7 ng /ml (range 0.4 – 3.5 ng /ml ), while the toxic group had a mean plasma level of 4.4 ± 0.9 ng /ml. It was concluded that the difference between the mean digoxin levels in toxic and nontoxic patients was significant, however, a certain degree of overlapping did exist. It was also concluded that the blood sample should be withdrawn at least six hours after the last dose of digoxin to correctly judge the plasma digoxin level.

Park et al [2] measured serum digoxin levels in a group of 129 adult patients, and found a mean digoxin level of 1.1 ± 0.1 (1 S E ) ng /ml for 108 nontoxic patients, and a mean level of 3.8 ± 0.5 ng /ml for 21 toxic patients. An overall accuracy of 89 percent was reported for the method in differentiating between toxic and nontoxic patients. Ninety percent of the nontoxic patients had serum digoxin levels below 2 ng /ml , while 86 percent of the toxic patients had levels above 2 ng /ml. Blood samples were drawn between 5 and 30 hours after the last digoxin dose.

In a review article, Smith and Haber[6] observed that despite the multiple variables known to influence cardiac response to digitalis glycosides, significantly higher mean serum digoxin levels were observed in toxic patients compared with nontoxic patients in nearly all studies published to date; the study of Engelman et al [17] being the only exception. Results on over 1,000 patients have shown that mean digoxin levels in patients with toxic manifestations are about two-fold higher than those of patients without toxicity. They point out that despite the significantly different mean levels, however, overlap has been observed in most series, and it must be emphasized that no arbitrary level can be chosen which clearly differentiates toxic from nontoxic serum digoxin concentrations. They conclude that serum digoxin levels are most useful when interpreted together with all other relevant variables in the clinical context.

## REAGENTS

The Digoxin CLASP RIA Kit is available in a 100-tube package. Each kit contains 5 vials of $^{125}I$ Digoxigenin (lyophilized, with a total activity of <0.45 microcurie per vial and containing buffers, a carrier, and a preservative); 6 vials of Digoxin Standard (1 ml each; 0 ng, 0.5 ng, 1.0 ng, 2.0 ng, 3.0 ng, and 5.0 ng with a preservative); 100 Digoxin Antibody Coated Tubes (with a binding agent); and 1 vial of Digoxin Control (1 ml in sheep serum with a preservative).

**WARNINGS**

For *In Vitro* Diagnostic Use. For Professional Use Only.

Note: This radioactive material may be received, acquired, possessed, and used only by physicians, clinical laboratories or hospitals and only for *in vitro* clinical or laboratory tests not involving internal or external administration of the material or the radiation therefrom to human beings or animals. Its receipt, acquisition, possession, use, and transfer are subject to the regulations and a general license of the U S Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

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*In vitro* clinical laboratory testing with the Digoxin CLASP RIA Kit requires only a general license from the U S Nuclear Regulatory Commission. The general license is issued to any physician, clinical laboratory, or hospital who obtains a validated registered U S N R C Form 483. This form must be submitted in triplicate to the U S N R C. The possessor of a general license is subject to the conditions and limitations under 10 CFR 31.11. (A specific license is available from the U S N R C for quantities larger than 200 microcuries.

**PRECAUTIONS**

The by-product material should be stored in the original shipping container or in a container providing equivalent radiation protection until used. Observe the following precautions in handling radioactive material: 1) There should be no pipetting by mouth; 2) There should be no smoking or eating while radioactive materials are being handled; 3) Cover hands with rubber gloves during and wash thoroughly after handling radioactive materials; 4) Wipe up spills quickly and thoroughly—add the contaminated materials to radioactive waste matter; 5) Solid waste can be stored until it decays. It may be discarded in the customary manner, after removing labeling. When the radioactive material used has a relatively short physical half-life, contaminated material may be stored in a specifically designated area in a covered metal or plastic container conspicuously marked with a radiation caution label. Monitor each piece of material for radioactivity and store until the decay can no longer be measured.

IMPORTANT NOTE: The commonly used bacteriostatic agent, sodium azide, that is used in most of the reagents supplied with the Digoxin CLASP RIA Kit, has been implicated in laboratory explosions when this material was disposed of through the laboratory plumbing system. To avoid the formation of highly explosive copper and lead azides, it is recommended that excess reagents and assay samples that are disposed of through the laboratory plumbing system be thoroughly flushed with large amounts of water.

## PREPARATION OF TEST REAGENTS

The $^{125}$I Digoxigenin Solution: Add 25 ml of distilled water to one vial of the lyophilized $^{125}$I Digoxigenin. Swirl gently until a clear solution is obtained. The $^{125}$I Digoxigenin Solution may be stored at 2° to 8° C or frozen, and prepared fresh each week.

**STORAGE**
The Digoxin Standards, the lyophilized $^{125}$I Digoxigenin, and the Digoxin Control must be stored below -10° C. The Digoxin Antibody Coated Tubes should be stored at 2° to 8° C.

### COLLECTION OF BLOOD SAMPLES FOR ANALYSIS

An appropriate quantity of blood should be collected from a peripheral vein using a standard blood collection tube. The requirement for an anticoagulant in the tube will depend on whether the test will be conducted on serum or plasma. Blood samples that are not processed on the day they are collected, should be stored under refrigeration. Serum or plasma samples should be frozen if not assayed within 48 hours of sample collection.[18]

There is considerable variability in the literature on the time at which the blood sample should be drawn following the last dose of digoxin, with intervals ranging from 3 to 48 hours being reported. Redfors[19] claims that an interval of 24 hours provided a steady state condition that gave the most reliable reflection of the digoxin concentration in the myocardium, and gave good day to day reproducibility. Most other investigators used intervals of 4 to 12 hours, with the general consensus being that an interval of at least six hours should be allowed for serum or plasma concentrations to reach a plateau. Regardless of the time interval used, it is strongly recommended that the time interval be recorded for each specimen drawn, and the digoxin level be expressed in ng /ml for the chosen time interval. It is self-evident that the greatest diagnostic accuracy will be attained by establishing toxic and nontoxic levels at a specific time interval. The same levels and time intervals should be used for subsequent diagnostic studies.

In addition to specifying the time interval between blood collection and the last digoxin dose, the patient history should be thoroughly scrutinized to determine if any diagnostic or therapeutic radioisotopes have been administered to the patient in the week or two immediately prior to the digoxin assay. It is also important for the assay request to specify the exact drug being used (i.e., digoxin, digitoxin, or digitalis), to avoid erroneous assay results.

There is no requirement that the patient be in the fasting state at the time of administration of the last digoxin dose or during the interval prior to collection of the blood sample.

RADIOIMMUNOASSAY TEST PROCEDURE
Materials Needed

In addition to the reagents and materials supplied with the Digoxin CLASP RIA Kits, the following equipment is required:

1. Well-type gamma scintillation counter
2. Refrigerator
3. Freezer
4. Controlled temperature water bath (37° C)
5. Pipettor and tips for 1.0 ml and 50 µl
6. Container for radioactive waste
7. Usual bench equipment including racks, graduates, and beakers of appropriate sizes to accommodate the quantities of reagents used in this test
8. Normal saline solution

Important
It will take approximately one hour for the frozen reagents to thaw upon removal from the carrier.

Repeated thawing and refreezing of the Digoxin Standard and Digoxin Control do not significantly affect test results. The Digoxin Standard and Control should be stored in their original containers, with their original closures. Do not transfer these reagents to another container or change the closure supplied with the vials.

Assay results will be nonlinear if there is deviation from the recommended 50 µl sample size.

Reagents from individual kits should not be intermixed with reagents from other kits.

Use appropriate radiation precautions in handling, identifying, and discarding all radioactive material since minute amounts of radioactivity remain on components used in the test.

Procedure
The procedure described below is based on performance of duplicate analyses on all samples assayed. Read entire procedure before starting test.

1. Mark a series of Digoxin Antibody Coated Tubes with the numbers 1 through 16. The first 12 tubes are required for the standard curve, tubes 13 and 14 are required for the Digoxin Control Serum, and tubes 15 and 16 are required for the assay of one clinical sample. Two additional tubes should be used for each additional clinical sample to be assayed.

2. Add the following quantities of Digoxin Standard to the respective tubes: Tubes 1 and 2 - 50 µl of 0 ng Standard; Tubes 3 and 4 - 50 µl of 0.5 ng Standard; Tubes 5 and 6 - 50 µl of 1.0 ng Standard; Tubes 7 and 8 - 50 µl of 2.0 ng Standard; Tubes 9 and 10 - 50 µl of 3.0 ng Standard; Tubes 11 and 12 - 50 µl of 5.0 ng Standard.

3. Add 50 µl of Digoxin Control to Tubes 13 and 14.

4. Add 50 µl of the clinical sample to Tubes 15 and 16.

The composition of the 16 tubes is summarized below:

| Tube # | Sample Added | Digoxin Content (ng/ml) |
|---|---|---|
| 1 and 2 | 50 µl 0 ng Std. | 0 |
| 3 and 4 | 50 µl 0.5 ng Std. | 0.5 |
| 5 and 6 | 50 µl 1.0 ng Std. | 1.0 |
| 7 and 8 | 50 µl 2.0 ng Std. | 2.0 |
| 9 and 10 | 50 µl 3.0 ng Std. | 3.0 |
| 11 and 12 | 50 µl 5.0 ng Std. | 5.0 |
| 13 and 14 | 50 µl Digoxin Control | See Label Assay |
| 15 and 16 | 50 µl Clinical Sample | Unknown |

5. Add 1.0 ml of $^{125}I$ Digoxigenin Solution to each tube.

6. Shake the test tube rack gently by hand to mix the contents of the tubes.

7. Allow the tubes to stand at room temperature (22° to 24° C) for one hour or, if more convenient, incubate at 37° C for 30 minutes. Equivalent results will be obtained with either set of incubation conditions.

8. At the end of the incubation period, decant or aspirate the contents of each tube and discard to radioactive waste.

9. Add 1 ml of normal saline to each tube. Decant or aspirate the saline wash solution and discard to radioactive waste.

10. Measure the radioactivity remaining in the empty tubes by counting in a standard well-type gamma scintillation counter (discriminator settings 20 to 50 keV). Subtract background counts and record net cpm (BOUND COUNTS) for each tube on the worksheet provided with each kit.

Results

1. Calculate the average BOUND COUNTS for the duplicate samples and record average values on the worksheet.

2. Calculate average $B/B_o$ for the duplicate samples using the following formula and record values on the worksheet:

$$B/B_o = \frac{\text{AVERAGE BOUND COUNTS (net cpm)}}{\text{AVG. BOUND COUNTS OF "0" ng Std. (net cpm)}}$$

Example: Background cpm: 250
Avg. BOUND COUNTS (Clinical sample) (gross cpm): 3750
Avg. BOUND COUNTS (0 ng Std) (gross cpm): 5150

$$B/B_o = \frac{3750 - 250}{5150 - 250}$$

$$= \frac{3500}{4900} = 0.71$$

3. Prepare a Standard Curve by plotting the calculated $B/B_o$ values against the log of the quantity of Digoxin Standard added to the respective tubes (i.e., tubes 1 through 12). See the sample Standard Curve depicted on the worksheet for guidance in plotting the data. Alternatively, the data may be plotted as $B/B_o$ against the linear value of the quantity of Digoxin Standard added. A sample Standard Curve is also provided for guidance in plotting this alternate type of curve. The Standard

Curves shown on the worksheet are provdided for guidance only, and should not be used for calculating Digoxin levels in clinical samples.

4. For individual laboratory quality control purposes, determine the quantity of Digoxin in the Digoxin Control Serum sample (tubes 13 and 14) by referring to the Standard Curve prepared above. The $B/B_o$ value will correspond to the specific quantity of Digoxin contained in the sample. The quantity measured should be in reasonably good agreement with the quantity specified on the Digoxin Control Serum label.

5. Determine the quantity of Digoxin in the clinical sample by referring to the Standard Curve prepared above. The $B/B_o$ for each sample will correspond to the specific quantity of Digoxin contained in the sample. The Digoxin concentration in the Digoxin Standards has been adjusted to correct for sample dilution, and will thus provide a direct indication of Digoxin concentration in the serum or plasma in terms of nanograms per milliliter (ng/ml). This value is read directly from the Standard Curve, with no further calculations required.

## EXPECTED VALUES

Since differences in laboratory techniques and other variations in technical factors will affect radioimmunoassay results, it is recommended that each laboratory establish its own range of nontoxic and toxic values. Using the Digoxin CLASP RIA Kit, the following results were obtained by two independent investigators studying nontoxic and toxic patients:

| Nontoxic Patients | Mean Digoxin Level (ng/ml) | Range of Values (ng/ml) |
|---|---|---|
| 117 patients | 1.13 ± 0.66 | 0 - 2.5 |
| 73 patients | 1.07 ± 0.48 | 0.3 - 2.2 |
| **Toxic Patients** | | |
| 17 patients | 3.91 ± 1.03 | 2.7 - 5.8 |
| 17 patients | 2.58 ± 0.41 | 2.2 ->5.0 |

In studies on the same patient samples, using the charcoal separation based Digoxin IMMUTOPE Kit, the nontoxic and toxic levels were similar to those reported above, but the Digoxin CLASP RIA Kit results were slightly lower in the nontoxic patients and slightly higher in the toxic patients. In general, the solid-phase Digoxin CLASP RIA Kit provides better separation of nontoxic and toxic patients than does the charcoal based Digoxin IMMUTOPE Kit.

PARAMETERS OF THE TEST PROCEDURE

The specificity and sensitivity of the test procedure depends on the specific characteristics of the antiserum provided in the individual kit shipments. With the antiserum provided in the Digoxin CLASP RIA Kit, crossreactivity with digitoxin is no more than 6 percent in the therapeutic range. This crossreactivity will generally be of little consequence as plasma from patients taking digitoxin should not be assayed with the Digoxin CLASP RIA Kit. The antibody crossreactivity with dihydrodigoxin (a nonbioactive metabolite) at the serum levels reported[20] will not significantly alter the test results. Human serum albumin in concentrations up to 70 mg/ml does not significantly affect test results.

The sensitivity of the assay system is a function of the avidity of the specific antiserum employed in the assay system. The sensitivity of the Digoxin CLASP RIA Kit based on the lowest detectable quantity of Digoxin is 0.1 ng/ml. The affinity constant for the antiserum is $1.1 \times 10^{10}$ liters/mole.

The reproducibility of test results is based on a great number of variables, not the least of which is the attention to detail exercised by the individual performing the text. Exercising a reasonable degree of care, reproducibility of Digoxin CLASP RIA Kit results have shown an intraassay coefficient of variation (CV) of up to 6.8 percent for a range of Digoxin levels. An interassay CV of up to 8.8 percent was observed for samples over the same range of concentrations.

Studies on the recovery from serum of added Digoxin have been conducted with the Digoxin CLASP RIA Kit over a wide range of Digoxin concentrations. The recoveries were essentially quantitative.

The wash step described in Step #9 of the procedure can be omitted if strict attention is given to complete and reproducible removal of the contents of the tube in Step #8. To facilitate such removal by the decantation technique, the lip of the inverted tube should be touched with a paper towel or tissue paper.

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Princeton, N.J. 08540

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Corp. Mark®

CAUTION
(Daisy)
RADIOACTIVE
MATERIAL

Medotopes®
(symbol)®

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Digoxin CLASP™ RIA Kit

Squibb Digoxin Radioimmunoassay Kit

For in vitro Diagnostic Use • WARNING: NOT FOR INJECTION

Not for Internal or External Use in Humans or Animals

Contains sufficient material for 100 tubes • See enclosed directions

CONTENTS:
5 vials $^{125}$I DIGOXIGENIN $<$ 0.45 microcuries/vial
6 bottles (1 ml ea.) DIGOXIN STANDARD (1 each of 0 ng, 0.5 ng, 1.0 ng, 2.0 ng, 3.0 ng and 5.0 ng/ml)
1 bottle (1 ml) DIGOXIN CONTROL
100 tubes DIGOXIN ANTIBODY COATED TUBES

ON RECEIPT STORE DIGOXIN ANTIBODY COATED TUBES AT 2° - 8° C
STORE BALANCE OF KIT BELOW -10° C

E. R. Squibb & Sons, Inc. Princeton, N.J. 08540
Made in U.S.A. C8113/G1310

SHIPPER LABEL

Expiration Date and Lot No. are added at the time of manufacture

85645

Corp Mark®

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RADIOACTIVE
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Digoxin CLASP™ RIA Kit

Squibb Digoxin Radioimmunoassay Kit

For in vitro Diagnostic Use • WARNING: NOT FOR INJECTION

Not for Internal or External Use In Humans or Animals

Contains sufficient material for 100 tubes

STORE BELOW -10° C • See enclosed directions

CONTENTS:
5 vials $^{125}$I DIGOXIGENIN
< 0.45 microcuries/vial
6 bottles (1 ml ea.) DIGOXIN STANDARD
(1 each of 0 ng, 0.5 ng, 1.0 ng,
2.0 ng, 3.0 ng and 5.0 ng/ml)
1 bottle (1 ml) DIGOXIN CONTROL

E. R. Squibb & Sons, Inc., Princeton, N.J. 085
Made in U.S.A. C5046/G1310

CARRIER LABEL

Expiration Date and Lot No. are added at the time of manufacture

CAUTION
(Daisy)
RADIOACTIVE MATERIAL

SQUIBB

$^{125}$I DIGOXIGENIN List G1310

For *in vitro* Diagnostic Use • See directions

WARNING: NOT FOR INJECTION

Not for Internal or External Use in Humans or Animals

Reconstitute with 25 ml distilled water

Store below -10° C. After reconstitution, store at 2° - 8° C or frozen. Do not use 7 days after reconstitution.

| | | |
|---|---|---|
| Total Act.: < 0.45 microcuries | As of | Noon E.S.T. |
| LOT NO.: | EXP. DATE | |

E. R. Squibb & Sons, Inc., Princeton, N.J. 08540
Made in U.S.A. C6208/G1310

VIAL LABEL

Assay date, Lot No. and Expiration Date are added at the time of manufacture

85645