Monobind Inc.
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AccuLite
CLIA Microwells

Digoxin (DIG) Test System
Product Code: 975-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Digoxin Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Chemiluminescence

2.0 SUMMARY AND EXPLANATION OF THE TEST

The clinical usefulness of the measurement of serum digoxin (DIG) is due to its low therapeutic ratio; a very small difference exists between therapeutic and toxic tissue levels. In addition, individuals may vary in their response to digoxin with an apparent increase in susceptibility to toxicity with age.[1]

The action of digoxin is to increase the force and velocity of myocardial contraction. This is necessary in the treatment of congestive heart failure and arrhythmias such as atrial fibrillation and atrial flutter.[2]

The myocardial concentrations of digoxin to serum levels remain relatively constant during normal renal function. This distribution ratio of digoxin is approximately 29 to 1 between the heart and serum.[3] Thus, monitoring digoxin therapy by measurement of serum levels is feasible from the pharmacological standpoint, since serum levels are related to tissue levels following post-absorption equilibration.[1] A practical and sensitive method of digoxin quantitation in serum is by enzyme immunoassay.

This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-digoxin conjugate is added, and then the reactants are mixed. A competition reaction results between the enzyme conjugate and the native digoxin for a limited number of antibody combining sites immobilized on the well.

After the completion of the required incubation period, the antibody bound enzyme-digoxin conjugate is separated from the unbound enzyme-digoxin conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce light.

The employment of several serum references of known digoxin concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with digoxin concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 7):
The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen.

Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the following equation:

$$^{Enz}Ag + Ag + Ab_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

Ab_{Btn} = Biotinylated Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{Btn}$ = Antigen-Antibody Complex
$^{Enz}AgAb_{Btn}$ = Enzyme-antigen Conjugate -Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.
$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin}_{cw} \Rightarrow$ immobilized complex
$\underline{Streptavidin}_{cw}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen con-centration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. Digoxin Calibrators – 1ml/vial - Icons A-F
Six (6) vials of serum reference for digoxin at concentrations of 0 (**A**), 0.25 (**B**), 0.5 (**C**), 1.0 (**D**), 2.0 (**E**) and 4.0 (**F**) ng/ml. A preservative has been added. Store at 2-8°C.

B. Digoxin Tracer Reagent– 6ml/vial - Icon Ⓔ
One (1) vial of Digoxin-Horseradish peroxidase (HRP) conjugate in a buffer with dye. A preservative has been added. Store at 2-8°C.

C. Digoxin Biotin Reagent – 6ml/vial - Icon ∇
One (1) vial of reagent contains anti-digoxin biotinylated rabbit serum conjugate in buffer, dye and preservative. Store at 2-8°C.

D. Light Reaction Wells – 96 wells –Icon ⇓
One 96-well microplate coated with 1.0 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml/vial - Icon
One (1) vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Signal A – 7ml/vial - Icon C[A]
One (1) vial contains luminol in a buffer. Store at 2-8°C.

G. Signal B – 7ml/vial - Icon C[B]
One (1) vial contains hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipettes capable of delivering 0.025ml (25µl) and 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Luminometer.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and taken with the usual precautions in the collection of venipuncture samples. The blood should be collected in a redtop (with or without gel additives) venipuncture tube. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
   Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at room temperature (2-30°C) for up to 60 days.
2. **Working Signal Reagent Solution -** Store at 2 - 8°C.
   Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1ml of B per two (2) eight well strips (a slight excess of solution is made). **Discard the unused portion if not used within 36 hours after mixing.** If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

Note: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.050 ml (50µl) of Digoxin Tracer Reagent to all the wells
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Add 0.050 ml (50µl) Digoxin Biotin Reagent to all wells
6. Swirl the microplate gently for 20-30 seconds to mix.
7. Cover and incubate for 30 minutes at room temperature.
8. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
9. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
10. Add 0.100 ml (100µl) of working signal reagent solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
    DO NOT SHAKE PLATE AFTER SIGNAL ADDITION
11. Incubate at room temperature for five (5) minutes in the dark.
12. Read the relative light units in each well with a Chemiluminescence microplate reader for 0.5-1.0 seconds. **The results should be read within 30 minutes after adding the working Signal Reagent.**

Note: For re-assaying specimens with concentrations greater than 4 ng/ml, pipet 0.0125ml (12.5µl) of the specimen and 0.0125ml (12.5µl) of the 0 serum reference into the sample well. Multiply the readout value by 2 to obtain the digoxin concentration.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of digoxin in unknown specimens.
1. Record the RLUs obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the RLUs for each duplicate serum reference versus the corresponding free testosterone concentration in pg/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of digoxin for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLUs (35157) intersects the standard curve at (1.16ng/ml) digoxin concentration (See Figure 1).

Note: Computer data reduction software designed for chemiluminescent assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

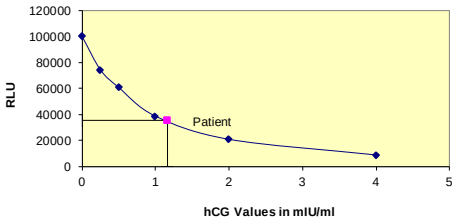
EXAMPLE 1

Sample I.D.	Well Number	RLUs	Mean RLUs	Value (ng/ml)
Cal A	A1	100032	100000	0
	B1	99967		
Cal B	C1	73049	74254	0.25
	D1	75459		
Cal C	E1	59836	60640	0.5
	F1	61444		
Cal D	G1	38113	38591	1.00
	H1	39069		
Cal E	A2	20364	20827	2.00
	B2	21291		
Cal F	C2	8511	8808	4.00
	D2	9104		
Patient	E2	35065	35157	**1.16**
	F2	35249		

* The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLU's of the calibrators have been normalized to 100,000 RLU's for the F calibrator

(greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light output.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The Dose Response Curve should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipeting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for AccuLite® CLIA procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (Boscato, LM, Stuart, MC. ''Heterophilic antibodies: a problem for all immunoassays'' *Clin. Chem*. 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. Certain disease states are known to increase a patient's susceptibility to digoxin toxicity.[4] The following are examples of such disease states hypokalaemia, hypothyroidism, renal Failure, and advanced Heart Disease.
8. A number of researchers have reported relatively high serum digoxin levels in infants. However, digoxin treated-children older than two years of age demonstrate serum digoxin levels more closely resembling adult values.[3]
9. Patients receiving simultaneous quinidine and digoxin therapy should be monitored closely.[6] Serum digoxin levels may rise to greater than twice the stabilized level within 24 hours after initiation of quinidine therapy and may remain higher for several days.
10. Patients receiving the diuretic furosemide may not display digoxin values that correspond to the clinical picture.[6] When furosemide and digitalis preparations are used concurrently, monitoring patients is desirable.[7]
11. Individuals on large doses of biotin supplements should discontinue use one day before blood draw in order to eliminate possible interferences.

13.0 EXPECTED RANGES OF VALUES

The usual therapeutic range of digoxin in adults is 0.5-2.0 ng/ml. However, there is an overlap of serum digoxin concentrations in groups of patients with and without clinical toxicity. A significant number of non-toxic patients have serum concentrations greater than 2.0 ng/ml and a correspondingly significant number of toxic patients have serum values in the range of 1.4-2.0 ng/ml.[8] Also, patients with supraventricular arrhythmias may require higher doses to control their cardiac rate: these patients' digoxin concentrations range from 2.0-4.0 ng/ml without clinical toxicity. For these reasons, the physician should make a definite clinical diagnosis after all clinical and laboratory findings have been evaluated.

TABLE I
Expected Values for the Digoxin Test System

Normal Adult	0.5 – 2.0 ng/mL

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the Digoxin AccuLite® CLIA test system were determined by analyses on three different levels of pool control sera. The number (N), mean values (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.%
Low	24	0.57	0.35	6.1
Normal	24	1.90	0.084	4.4
High	24	2.99	0.140	4.7

TABLE 3
Between Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.%
Low	10	0.53	0.05	9.4
Normal	10	1.82	0.11	6.0
High	10	3.12	0.17	5.4

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The Digoxin AccuLite® CLIA test system has an analytical sensitivity of 0.169 ng/ml. The sensitivity was ascertained by determining the variability of the '0' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum concentration.

14.3 Specificity

The cross-reactivity of the digoxin antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between doses of interfering substance to dose of digoxin needed to displace the same amount of tracer.

Substance	Cross Reactivity
Digoxin	1.000
Di-Acetyldigoxin	1.00
β-Mehtyldigoxin	1.00
α-Acetyldigoxin	1.00
Digitoxin	0.019
Digitoxigenin	0.017
Lanatoside A	0.016
Ouabain	0.001
Spironolactone	0.001
Prednisone	0.001
Pregnenolone	0.001
Digitoxose	0.001

15.0 REFERENCES

1. Doherty JE and Kane JJ, "Clinical Pharmacology of Digitalis Glycosides", *ANN REV MED*, 26, 159 (1975)
2. Butler VP, "Assays of Digitalis in blood", *PROG CARDOVASC DIS*, 14, 571 (1972).
3. Henry JB, Therapeutic Drug Monitoring and Toxicology", *CLINIAL DIGANOSIS and MANAGEMENT*, 1, 482 (1974).
4. Beller, GA, et al, *NEW ENG J MED*, 284, 989 (1979).
5. Swidler, G, *HANDBOOK of DRUG INTERACTIONS*, Wiley-Interscience, New York, 253 (1971).
6. Duron O., *RADIOASSAY NEWS* 2, 35 (1975).
7. Swidler G, "HANDBOOK of drug interactions", *Wiley-Interscience*, New York, p. 150 (1979).
8. Butler VP, and Lindinbaum J, "Serum Digitalis Measurements in the Assessment of Digitalis Resistence and Sensitivity", *AM J MED*, 58, 460 (1975).

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Size		96(A)	192(B)
Reagent (fill)	**A)**	**1ml set**	**1ml set**
	B)	**1 (6ml)**	**2 (6ml)**
	C)	**1 (6ml)**	**2 (6ml)**
	D)	**1 plate**	**2 plates**
	E)	**1 (20ml)**	**1 (20ml)**
	F)	**1 (7ml)**	**2 (7ml)**
	G)	**1 (7ml)**	**2 (7ml)**

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Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

- IVD — In Vitro - Diagnostic Medical Device
- 2° 8° — Temperature Limitation Storage Condition (2-8°C)
- Consult Instructions for Use
- REF — Catalogue Number
- Σ — Contains Sufficient Test for Σ
- LOT — Batch Code
- Used By (Expiration Day)
- Date of Manufacturer
- Manufacturer
- EC REP — Authorized Rep in European Country
- CE — European Conformity