DRI® Digoxin Assay

Intended Use
The DRI® Digoxin Assay is intended for the quantitative determination of digoxin in human serum or plasma.

Summary and Explanation of the Test
Digitalis is known to have the ability to increase the force and velocity of myocardial contraction.1 Digoxin is one of the most commonly used forms of digitalis in the treatment of congestive heart failure and arrhythmia such as atrial fibrillation and atrial flutter. The therapeutic range of digoxin is narrow. Furthermore, individual differences in drug absorption, distribution, metabolism, and excretion as well as factors such as concurrent use of other drugs and illness can also alter the serum concentration in response to a given dosage. Monitoring the serum digoxin concentration is the most effective means of improving the drug efficacy and minimizing the risk of toxicity.1,2

The current Digoxin Assay is a homogeneous immunoassay based on the measurement of the extent of light scattering, which results from the aggregation of activated microparticles. The activated microparticles are immobilized with digoxin and are allowed to react with a mixture of patient serum sample and anti-digoxin antibody solution. In the absence of digoxin from the sample, the digoxin immobilized on the microparticles is bound by the digoxin antibody. If the sample contains digoxin, aggregation of these microparticles is partially inhibited. This phenomenon creates a relationship between the digoxin concentration in the sample and the extent of the microparticles aggregation. The extent of the particle aggregation can be monitored spectrophotometrically by determining the change in light scattering or absorbance.

Reagents
Antibody Reagent. Contains monoclonal anti-digoxin antibody in a buffer solution with sodium azide as a preservative.

Microparticles Reagent. Contains digoxin coated microparticles suspension in an aqueous solution with sodium azide as a preservative.

Calibrators
DRI Digoxin Calibrator A (1 x 5 mL): Contains 5 mL buffer solution with sodium azide as a preservative.
DRI Digoxin Calibrator B (1 x 2 mL): Contains Digoxin in buffer solution with sodium azide as a preservative.
DRI Digoxin Calibrator C (1 x 2 mL): Contains Digoxin in buffer solution with sodium azide as a preservative.
DRI Digoxin Calibrator D (1 x 2 mL): Contains Digoxin in buffer solution with sodium azide as a preservative.
DRI Digoxin Calibrator E (1 x 2 mL): Contains Digoxin in buffer solution with sodium azide as a preservative.
DRI Digoxin Calibrator F (1 x 2 mL): Contains Digoxin in buffer solution with sodium azide as a preservative.

(Refer to the assignment addendum included in the kit for the actual calibrator values).

Precautions and Warnings
DANGER: DRI Digoxin Assay contains ≥6.4% Choline salt, ≥5.0% Bic-Tris, ≥1.0% Antibody, ≤3.0 Human Serum Albumin (HSA), & ≤1.9% Carbodiimide Hydrochloride.
H315 - Causes skin irritation.
H317 - May cause allergic skin reaction.
H319 - Causes serious eye irritation.
H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Avoid breathing mist or vapor. Wash hands thoroughly after handling. Contaminated work clothing should not be allowed out of the workplace. Wear protective gloves/eye protection/respiratory protection. If on skin: Wash with plenty of soap and water. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Specific treatment (see First Aid information on product label and/or Section 4 of the SDS). If skin irritation or rash occurs: Get medical advice/attention. If eye irritation persists: Get medical advice/attention. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. Wash contaminated clothing before reuse. Dispose of contents/container to location in accordance with local/regional/national/international regulations.

This test is for in vitro diagnostic use only. The reagents are harmful if swallowed.

Reagents used in the assay components contain sodium azide. Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Get immediate medical attention for eyes, or if ingested. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up. Clean exposed metal surfaces with 10% sodium hydroxide.

Do not use the reagents beyond their expiration dates.

When evaluating patient results, one should consider other clinical factors and symptoms.

Reagent Preparation and Storage
The reagents are ready for use. No reagent preparation is required. All assay components, when stored properly at 2-8°C, are stable until the expiration date indicated on the label.

Specimen Collection and Handling
Pharmacokinetic factors, such as dosage form, mode of administration, concomitant drug therapy as well as the patient’s clinical condition may influence the correct time of sample collection.2,3 For reliable interpretation of results, a serum specimen should be collected 6 to 8 hours following the last oral dose of digoxin. Either serum or heparin and EDTA treated plasma samples can be used with the assay. Samples may be stored refrigerated at 2-8°C for up to 7 days or frozen (-20°C) for up to 6 months. For lipemic samples, avoid collecting lipids from the top portion.

Handle all serum or plasma specimens as if they were potentially infectious.

Assay Procedure
Analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring light scattering or absorbance and timing the reaction accurately may be used to perform this assay. Before performing the assay, refer to the analyzer-specific protocol sheet, which contains parameters and/or additional instructions for use.

Quality Control and Calibration
Good laboratory practice suggests the use of control specimens to ensure proper assay performance. Controls results must fall within the established ranges, as determined by your laboratory. If results fall outside of the established ranges, assay results are invalid. All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Results and Expected Values
The therapeutic efficacy and toxic effects of digoxin are closely related to the serum digoxin concentration. Most patients achieve a satisfactory therapeutic response when the serum digoxin concentration is between 0.9 and 2.0 ng/mL. Toxic manifestations commonly occur at concentrations greater than 2.0 ng/mL. However, some patients with supraventricular arrhythmia may require higher doses of digitalis to control their cardiac rate. These patients may have serum digoxin concentrations higher than 2.0 ng/mL without evidence of clinical toxicity. In addition, a patient’s clinical conditions, such as age, kidney function, thyroid status, and severity of heart disease and concomitant drug therapy as well as time of specimen collection may affect the relationship between digoxin serum concentration and clinical response. Therefore, when evaluating patient results one should consider other clinical factors and symptoms.

Limitations
1. The test is designed for use with serum or plasma only and not for whole blood.
2. Patient samples with digoxin concentration greater than 5 ng/mL can be diluted with negative calibrator and re-assayed. The correct digoxin concentration is then obtained by multiplying the assay result by the dilution factor.
3. The reagents are provided as a matched set. Do not interchange reagents from kits with different lot numbers.
4. Fab anti-digoxin (Digibind) in serum of patients under therapy for digoxin toxicity may interfere with the assay!2
5. Digoxin-like immunoreactive factors (DLIF), found in the serum of patients with renal or hepatic failure, neonates and pregnant women, have been reported to cause elevated digoxin immunoassay results.2,5
6. For diagnostic purposes, interfering heterophile antibodies occur at low frequency in the general population. These antibodies can cause auto-agglutination of the microparticle reagent leading to erroneous results that may be unexpectedly low or unexpectedly high. An erroneous result could lead to incorrect patient management; incorrect patient management could potentially cause serious injury or death. Test results should not be used in isolation to make patient management decisions. Results should always be assessed in conjunction with the patient’s medical history, clinical examinations, and other clinicopathological findings. An alternative test method should be used to confirm results when results are inconsistent with clinical expectations.
Typical Performance Characteristics

The following typical performance data were generated with a Hitachi 717 analyzer. Laboratories with different analyzers should establish their own performance characteristics.

Precision

The within-run and run-to-run precision were evaluated using three levels of control sera.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Within-run (n = 20)</th>
<th>Run-to-run (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD (ng/mL)</td>
<td>% CV</td>
</tr>
<tr>
<td>Control 1</td>
<td>0.5 ± 0.05</td>
<td>10.0</td>
</tr>
<tr>
<td>Control 2</td>
<td>1.5 ± 0.04</td>
<td>2.7</td>
</tr>
<tr>
<td>Control 3</td>
<td>3.5 ± 0.08</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Sensitivity

The lowest concentration that can be differentiated from the negative calibrator with 95% confidence, is 0.1 ng/mL.

Accuracy

One hundred and twelve clinical samples with digoxin concentration ranging from 0.2 to 3.8 ng/mL were assayed with the DRI Digoxin Assay (y) and a commercial fluorescence polarization assay (x). A correlation with a linear regression equation of $y = 1.0 \times + 0.01$ and a correlation coefficient of 0.97 were obtained.

Specificity

Cross-reactivity of the assay with other cardiac glycosides and their metabolites were studied by spiking pooled normal human serum with the cross-reactants and the resulting samples were assayed for equivalent digoxin concentration. The following table summarizes the results with these cardiac glycosides:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration Tested (ng/mL)</th>
<th>% Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digoxigenin</td>
<td>50</td>
<td>3.6</td>
</tr>
<tr>
<td>Digoxigenin Bisdigitoxoside</td>
<td>5</td>
<td>108.0</td>
</tr>
<tr>
<td>Digoxigenin Monodigitoxoside</td>
<td>5</td>
<td>82.0</td>
</tr>
<tr>
<td>Digitoxin</td>
<td>50</td>
<td>4.4</td>
</tr>
<tr>
<td>Digitoxigenin</td>
<td>500</td>
<td>0.7</td>
</tr>
<tr>
<td>D-(+)-Digitoxose</td>
<td>10,000</td>
<td>0.0</td>
</tr>
<tr>
<td>Gitoxin</td>
<td>150</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Comounds with similar chemical structure or compounds used concurrently with digoxin, as well as various endogenous hormones were tested for cross-reactivity in the assay. The following table summarizes the concentration of each compound tested which gave a cross-reactivity result less than the sensitivity of the assay.

<table>
<thead>
<tr>
<th>Compound</th>
<th>µg/mL</th>
<th>Compound</th>
<th>µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>1000</td>
<td>Phenobarbital</td>
<td>100</td>
</tr>
<tr>
<td>Cortisone</td>
<td>10</td>
<td>Phenytoin</td>
<td>100</td>
</tr>
<tr>
<td>Cortisol</td>
<td>10</td>
<td>Prednisolone</td>
<td>10</td>
</tr>
<tr>
<td>Dehydroisoandrosterone</td>
<td>10</td>
<td>Prednisone</td>
<td>10</td>
</tr>
<tr>
<td>Estriol</td>
<td>10</td>
<td>Procainamide</td>
<td>100</td>
</tr>
<tr>
<td>Furosemide</td>
<td>50</td>
<td>Progesterone</td>
<td>10</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>100</td>
<td>Propranolol</td>
<td>100</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>100</td>
<td>Quinidine</td>
<td>100</td>
</tr>
<tr>
<td>11-OH-progesterone</td>
<td>10</td>
<td>Secobarbital</td>
<td>100</td>
</tr>
<tr>
<td>17-OH-progesterone</td>
<td>10</td>
<td>Spironolactone</td>
<td>10</td>
</tr>
<tr>
<td>Ouabain Octahydrate</td>
<td>0.15</td>
<td>Testosterone</td>
<td>5</td>
</tr>
</tbody>
</table>

Clinical serum samples spiked with hemoglobin (up to 800 mg/dL), lipid (up to 1128 mg/dL) and bilirubin (up to 31 mg/dL) were found not to interfere with the digoxin concentration determined by this assay.

References


Glossary:

http://www.thermofisher.com/symbols-glossary