VIDAS® Digoxin (DIG)

IVD

VIDAS® Digoxin is an automated quantitative test for use on the VIDAS family instruments, for the immunoenzymatic determination of digoxin in human serum or plasma (lithium heparin or EDTA) using the ELFA (Enzyme Linked Fluorescent Assay). It is intended for use in monitoring treatment to ensure appropriate therapy.

SUMMARY AND EXPLANATION

Digoxin is one of the cardiac glycosides which share common specific effects on the myocardium (1). It is the cardiac glycoside most widely used in the treatment of heart failure as well as certain disturbances of cardiac rhythm (2). However, the therapeutic use of digoxin is limited by its narrow therapeutic range, with toxic effects generally seen at concentrations in plasma or serum above 2 ng/ml (3). These toxic side effects can include anorexia, nausea, vomiting, and arrhythmias (1, 4, 5).

The therapeutic window of digoxin must be closely monitored, taking into account individual sensitivity and associated conditions. The selected dosage must be based upon clinical assessment of the patient. Patients with renal insufficiency require lower than usual doses of digoxin (1, 6).

Sera should be collected 6-8 hours after a dose is administered to allow adequate time for equilibration of digoxin levels between serum and tissue. Monitoring serum digoxin levels can provide important information to the clinician to help achieve optimal therapeutic value to the patient.

PRINCIPLE

The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA).

The Solid Phase Receptacle (SPR®) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips.

All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.

The sample is transferred into the well containing digoxin conjugated with alkaline phosphatase. The digoxin present in the sample competes with the digoxin-alkaline phosphatase conjugate for binding with the rabbit anti-digoxin antibodies coated on the SPR. Wash steps remove unbound conjugate.

During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of digoxin present in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

CONTENT OF THE KIT (60 TESTS):

60 DIG strips	STR	Ready-to-use.
60 DIG SPRs 2 x 30	SPR	Ready-to-use. Interior of SPRs coated with rabbit anti-digoxin polyclonal immunoglobulins.
DIG control 1 x 2 ml (liquid)	C1	Ready-to-use Human serum* + digoxin + 1 g/l sodium azide. MLE data indicate the confidence interval in ng/mL ("Control C1 Dose Value Range").
DIG Calibrator 1 x 4 ml (liquid)	S1	Ready-to-use. Delipidated and defibrinated human plasma* + digoxin + 1 g/l sodium azide. MLE data indicate the concentration in ng/mL ("Calibrator (S1) Dose Value") and the confidence interval in “Relative Fluorescence Value” ("Calibrator (S1) RFV Range").
DIG diluent 1 x 1 ml (liquid)	R1	Ready-to-use Delipidated and defibrinated human plasma* + 1 g/l sodium azide.
Specifications for the factory master data required to calibrate the test: • MLE data (Master Lot Entry) provided in the kit, or • MLE bar code printed on the box label.		
1 Package insert provided in the kit or downloadable from www.biomerieux.com/techlib		

* This product has been tested and shown to be negative for HBs antigen, antibodies to HIV1, HIV2 and HCV. However, since no existing test method can totally guarantee their absence, this product must be treated as potentially infectious. Therefore, usual safety procedures should be observed when handling.

The SPR

The interior of the SPR is coated during production with rabbit anti-digoxin polyclonal immunoglobulins. Each SPR is identified by the DIG code. Only remove the required number of SPRs from the pouch and **carefully reseal the pouch after opening**.

The strip

The strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorometric reading is performed. The wells in the center section of the strip contain the various reagents required for the assay.

Description of the VIDAS Digoxin strip

Wells	Reagents
1	Sample well
2 - 3 - 4	Empty wells
5	Conjugate: digoxin conjugated to alkaline phosphatase + 1 g/l sodium azide (400 µl)
6	Empty wells
7 - 8 - 9	Wash buffer: TRIS buffered saline (0.05 mol/l, pH 7.4) + 1 g/l sodium azide (600 µl)
10	Cuvette with substrate: 4-Methyl-umbelliferyl phosphate (0.6 mmol/l) + diethanolamine* (DEA) (0.62 mol/l or 6.6%) pH 9.2 + 1 g/l sodium azide (300 µl).

* Signal Word: **DANGER**

Hazard statement

H318 : Causes serious eye damage.

Precautionary statement

P280 :Wear protective gloves/protective clothing/eye protection/face protection.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

For further information, refer to the Material Safety Data Sheet.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Pipette with disposable tips to dispense 100 µl.
- Powderless disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User's Manual.
- VIDAS family instrument.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only.**
- **This kit contains products of human origin. No known analysis method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (see Laboratory biosafety manual - WHO - Geneva - latest edition).**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
- Do not use the SPRs if the pouch is pierced.
- Do not use visibly deteriorated STRs (damaged foil or plastic).
- Do not use reagents after the expiration date indicated on the label.
- Do not mix reagents (or disposables) from different lots.
- Use **powderless** gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.
- Kit reagents contain sodium azide which can react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.
- The substrate in well 10 contains an irritant agent (6.6% diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.
- Spills should be wiped up thoroughly after treatment with liquid detergent or a solution of household bleach containing at least 0.5% sodium hypochlorite. See the User's Manual for cleaning spills on or in the instrument. Do not autoclave solutions containing bleach.
- The instrument should be regularly cleaned and decontaminated (see the User's Manual).

STORAGE CONDITIONS

- Store the VIDAS® Digoxin kit at 2–8°C.
- **Do not freeze reagents.**
- **Store all unused reagents at 2–8°C.**
- After opening the kit, check that the SPR pouch is correctly sealed and undamaged. If not, do not use the SPRs.
- **Carefully reseal the pouch with the desiccant inside after use to maintain stability of the SPRs and return the complete kit to 2–8°C.**

- If stored according to the recommended conditions, all components are stable until the expiration date indicated on the label.

SPECIMENS

Specimen type and collection:

Serum or plasma (Collected in a tube with lithium heparin or EDTA). Do not use heat-inactivated sera.
Hemolysis, lipemia, bilirubinemia and protein concentration have not been found to significantly influence this assay. See "**INTEFERENCE STUDIES"**.
If necessary, clarify samples by centrifugation.
It is recommended not to use samples that are clearly hemolyzed, lipemic or icteric and, if possible, to collect a new sample.

Specimen stability

Sera may be stored on the clot for up to 24 hours at 2–8°C. Sera removed from the clot and plasma samples can be stored at 2–8°C in stoppered tubes for up to 2 days. If longer storage is required, freeze samples at -25 ± 6°C for up to 2 months.
Avoid successive freezing and thawing.

INSTRUCTIONS FOR USE

For complete instructions, see the User's Manual.

Reading Master lot data

Before each new lot of reagents is used, enter the specifications (or factory master data) into the instrument using the master lot entry (MLE) data.

If this operation is not performed **before initiating the tests**, the instrument will not be able to print results.

Note: the master lot data need only be entered once for each lot.

It is possible to enter MLE data **manually or automatically** depending on the instrument (refer to the User's Manual).

Calibration

Calibration, using the calibrator provided in the kit, must be performed each time a new lot of reagents is opened, after the master lot data have been entered. Calibration should then be performed every 14 days. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf-life of the kit.
The calibrator, identified by S1, must be tested in **triplicate** (see User's Manual). The calibrator value must be within the set RFV "Relative Fluorescence Value" range. If this is not the case, recalibrate.

Procedure

1. **Only remove the required reagents from the refrigerator and allow them to come to room temperature for at least 30 minutes.**
2. Use one "DIG" strip and one "DIG" SPR for each sample, control or calibrator to be tested. **Make sure the storage pouch has been carefully resealed after the required SPRs have been removed.**
3. The test is identified by the "DIG" code on the instrument. The calibrator must be identified by "S1", and tested in **triplicate**. If the control is to be tested, it should be identified by "C1".
4. Mix the calibrator, control and samples using a vortex-type mixer (for serum or plasma separated from the pellet).
5. **For this test, the calibrator, control, and sample test portion is 100 µl.**
6. Insert the "DIG" SPRs and "DIG" strips into the instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent Strips match.
7. Initiate the assay as directed in the User's Manual. All the assay steps are performed automatically by the instrument.
8. Restopper the vials and return them to 2–8°C after pipetting.
9. The assay will be completed within approximately 20 minutes. After the assay is completed, remove the SPRs and strips from the instrument.
10. Dispose of the used SPRs and strips into an appropriate recipient.

RESULTS AND INTERPRETATION

Once the assay is completed, results are analyzed automatically by the computer. Fluorescence is measured twice in the Reagent Strip's reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR is introduced into the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The RFV (Relative Fluorescence Value) is calculated by subtracting the background reading from the final result. This calculation appears on the result sheet.
The results are automatically calculated by the instrument using calibration curves which are stored by the instrument (4-parameter logistic model). The concentrations are expressed in ng/ml.
The range of results for the VIDAS Digoxin assay is 0.2–5.0 ng/ml.
Samples with results less than 0.2 ng/ml are reported as < 0.2 ng/ml.
Samples with digoxin concentrations greater than 5 ng/ml should be retested after being diluted 1:2 in the DIG diluent (R1). Multiply the result by the dilution factor to obtain the sample concentration.

A report is printed which records:
- the type of test performed,
- the sample identification,
- the date and time,
- the lot number and the expiration date of the reagent kit being used,
- each sample's RFV and digoxin concentration.

Interpretation of test results should be made taking into consideration the patient's history.

QUALITY CONTROL

A control is included in each VIDAS® Digoxin kit.
This control must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. Each calibration must also be checked using this control. The instrument will only be able to check the control value if it is identified by C1.
Results cannot be validated if the control value deviates from the expected values.
Each laboratory must follow their regulatory guidelines for quality control.
Note: The precision data and the data collected in establishing the master curve enable the validation of samples tested singly (see Performance).

Note

It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.

LIMITATIONS OF THE METHOD

Serum from pregnant women, neonates, and patients with liver or kidney failure may contain digoxin-like immunoreactive factors (DLIF) which can cause elevated serum digoxin levels (6).
Interference may be encountered with certain sera containing antibodies directed against reagent components. For this reason, assay results should be interpreted taking into consideration the patient's history.

RANGE OF EXPECTED VALUES

Therapeutic range 0.6–1.2 ng/ml (7)

Toxic range > 2 ng/ml (3)

(1 x nmol/l = 1 x 1.28 ng/ml)
Because the therapeutic range can vary between individual patients, the expected values are provided only as a guideline. Assay results should be interpreted with clinical signs and symptoms as well as observing for toxicity.

PERFORMANCE

Studies performed using VIDAS Digoxin gave the following results:

Measurement range

The measurement range of the VIDAS Digoxin kit is: 0.2–5 ng /ml.

Detection limit

Defined as the smallest concentration of digoxin which is significantly different from the zero concentration with a probability of 95%: **0.2 ng/ml.**

Immunological specificity

The antibody used in the VIDAS Digoxin assay was tested for cross-reactivity against a number of compounds. The results in the table below are represented as a cross-reactivity percentage.

Tested compound	Concentration tested (ng/ml)	Cross-reactivity percentage (%)
Digoxigenin bis Digitoside	1.56	92.0
Deacetyllanatoside C	1.56	39.0
Lanatoside C	1.56	55.0
Digoxigenin	1.56	141.0
Digitoxigenin	25.0	2.7
Digitoxin	25.0	1.6

The following substances were tested at a concentration of 25 ng/ml and showed less than 1% cross-reactivity: Quinidine, Cortisone, Canrenoic acid, Corticosterone, Phenytoin in salt, Ouabain, Gitoxin, Procainamide, Estriol, Prednisolone, DHEA standard, Testosterone (at a concentration of 20 ng/ml), Spironolactone, Progesterone, Prednisone, Cholesterol, Furosemide, Hydrocortisone, 11 ß hydroxyprogesterone, 17 □ hydroxyprogesterone.

Precision

Intra-assay reproducibility

Five samples were tested 30 times in the same run.

Sample	1	2	3	4	5
Mean concentration (ng/ml)	0.32	0.75	1.15	1.77	3.98
% CV	14.0	6.7	6.7	5.7	5.1

Inter-assay reproducibility

Five serum samples were tested in 14 different runs on the same VIDAS® instrument over a 7-week period (recalibration was performed every 14 days as described in the User's Manual).

Sample	1	2	3	4	5
Mean concentration (ng/ml)	0.30	0.83	1.16	1.77	3.70
% CV	10.2	3.9	6.5	2.1	2.0

Inter-instrument inter-assay reproducibility

Five serum samples were tested singly in 8 different runs on different VIDAS instruments.

Sample	1	2	3	4	5
Mean concentration (ng/ml)	0.32	0.78	1.14	1.75	4.03
% CV	15.6	11.1	5.8	7.1	4.8

Accuracy – Dilution test

Three samples were diluted in digoxin free serum and tested in duplicate in 3 runs. The measured mean concentrations compared to the expected mean concentrations are shown as the mean recovery percentages in the table below.

Sample	Dilution factor	Expected concentration (ng/ml)	Mean measured concentration (ng/ml)	Mean recovery percentage (%)
1	1/1	4.66	4.66	100
	1/2	2.33	2.30	99
	1/4	1.16	1.11	96
2	1/1	3.71	3.71	100
	1/2	1.85	1.85	100
	1/4	0.92	0.87	94
3	1/1	1.75	1.75	100
	1/2	0.87	0.81	93
	1/4	0.43	0.39	91

Recovery test

Three samples were spiked with known quantities of digoxin (ng/ml) and tested singly in 3 different runs. The measured mean concentration compared to the expected mean concentration is expressed as a mean recovery percentage.

Sample	Amount spiked (ng/ml)	Expected mean concentration (ng/ml)	Measured mean concentration(ng/ml)	Mean recovery percentage (%)
1	0	0.20	0.20	100.0
	0.5	0.70	0.92	131.4
	1.0	1.20	1.27	105.8
	2.0	2.20	2.10	95.4
	4.0	4.20	3.65	87.0
	5.0	5.20	4.42	85.0
2	0	1.80	1.80	100.0
	0.5	2.30	2.51	109.1
	1.0	2.80	2.71	96.8
	2.0	3.80	3.45	90.8
	4.0	5.80	4.81	83.0
	5.0	6.80	> 5.00	-
3	0	3.96	3.96	100.0
	0.5	4.46	4.73	106.0
	1.0	4.96	4.88	98.4
	2.0	5.96	> 5.00	-
	4.0	7.96	> 5.00	-
	5.0	8.96	> 5.00	-

Comparison with another test method

Serum samples with digoxin concentrations ranging from 0.2–4 ng/ml were tested using the VIDAS Digoxin assay (Y) and 3 other commercially available assays (X). The results of the correlations for these samples are shown in the table below:

	Number of samples	Equation of the line	Correlation coefficient
FPIA	157	y = 1.01x – 0.10	0.97
EIA 1	157	y = 0.92x + 0.06	0.95
EIA 2	153	y = 0.86x + 0.05	0.88

INTERFERENCE STUDIES

Method of collection

Blood samples were collected from 26 patients. For each patient, 6 specimens were collected at the same time: in a plain glass tube; in a tube with separating gel; in a tube with EDTA-Na, in a tube with EDTA-K; in a tube with sodium citrate and in a lithium heparinized tube respectively. Each sample collected was tested in duplicate and sera from the same donor were tested in the same run. The plain glass tube was the reference to which the other methods were compared. No significant difference was observed with any of the specimen collection devices tested.

Heparin

Three pools of human serum were spiked with increasing quantities of lithium heparin.

		Amount of sodium heparin spiked (mg/ml)			
		0	0.5	5	50
Digoxin (ng/ml)	Pool 1	0.37	0.33	0.38	0.37
	Pool 2	0.89	0.81	0.73	0.84
	Pool 3	3.07	2.91	3.08	2.83

EDTA

Three pools of human serum were spiked with increasing quantities of EDTA.

		Amount of EDTA spiked (mg/ml)			
		0	1	5	10
Digoxin (ng/ml)	Pool 1	0.28	0.31	0.36	0.37
	Pool 2	0.68	0.85	0.79	0.94
	Pool 3	3.07	3.08	3.03	2.97

Hemoglobin

Seven aliquots of digoxin-free human sera were spiked with increasing quantities of hemoglobin obtained from a lysate of human red blood cells. Each of the aliquots was split into 3 pools and spiked with different amounts of digoxin.

		Amount of hemoglobin spiked (mg/dl)						
		0	12.5	25.0	50.0	100.0	500.0	1000.0
Digoxin (ng/ml)	Pool 1	0.37	0.30	0.23	0.26	0.28	0.28	0.24
	Pool 2	0.79	0.98	0.76	0.96	0.77	0.71	0.76
	Pool 3	2.87	3.18	3.42	3.67	3.21	3.13	2.92

Lipids

Three pools of human sera were spiked with increasing quantities of triglycerides.

		Amount of triglycerides spiked (mg/dl)				
		0	50	100	150	200
Digoxin (ng/ml)	Pool 1	0.49	0.35	0.32	0.34	0.36
	Pool 2	0.88	0.78	0.88	0.78	0.78
	Pool 3	3.08	3.05	3.02	2.94	3.02
Apparence		Clear	Opalescent	Turbid		

Bilirubin

Three pools of human sera were spiked with increasing quantities of bilirubin.

		Amount of bilirubin spiked (mg/dl)					
		0	2	5	10	15	30
Digoxin (ng/ml)	Pool 1	0.28	0.26	0.28	0.33	0.30	0.36
	Pool 2	0.69	0.77	0.81	0.84	0.75	0.97
	Pool 3	2.90	2.88	3.02	2.96	3.18	3.09

Protein

Three serum samples with known protein concentrations were spiked with defined quantities of digoxin.

	Sample 1 (22.4 g/l protein)	Sample 2 (78.5 g/l protein)	Sample 3 (132 g/l protein)
Expected concentration (ng/ml)	Measured concentration (ng/ml)	Measured concentration (ng/ml)	Measured concentration (ng/ml)
0.45	0.35	0.43	0.61
0.88	0.72	0.75	0.97
1.74	1.62	1.56	1.79

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

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INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalog number
IVD	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by date
LOT	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Date of manufacture

WARRANTY

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REVISION HISTORY

Change type categories :

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

Note: *Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release date	Part Number	Change Type	Change Summary
2015/01	07219L	Administrative	INDEX OF SYMBOLS REVISION HISTORY
		Technical	CONTENT OF THE KIT (60 TESTS) WARNINGS AND PRECAUTIONS
2015/06	07219M	Technical	CONTENT OF THE KIT (60 TESTS) INSTRUCTIONS FOR USE

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