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Electronic Instruction For Use: version

270_5

ORG 270 25-OH Vitamin D_3/D_2

INTENDED PURPOSE

25-OH Vitamin D_3/D_2 for Alegria® is an ELISA based test system intended for the quantitative measurement of the total concentration of 25-(OH)-Vitamin D_2 and 25-(OH)-Vitamin D_3 in human serum or plasma samples (EDTA plasma, heparin plasma, citrate plasma). This product is intended for professional in vitro diagnostic use only.
The concentration of 25-OH vitamin D is an indicator of the vitamin D supply in the body. Concentrations of 25-OH vitamin D over 20 ng/ml are considered to indicate a sufficient supply of vitamin D; values of 12-20 ng/ml indicate a lack of vitamin D; concentrations below 12 ng/ml indicate severe vitamin D deficiency. The level of 25-OH vitamin D correlates with the clinical symptoms of vitamin D deficiency.

SYMBOLS USED

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
24	Sufficient for ... determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Consult instructions for use
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
270_5	Electronic Instruction For Use: version

ALEGRIA TEST STRIPS	Alegria® Test Strips
WASH	Wash Buffer
SYSTEM FLUID	System Fluid
RTU	Ready to use

PRINCIPLE OF THE TEST

The Alegria® assay features barcoded 8-well-microstrips, called Alegria® Test Strips. Each strip is designed for a single determination of one patient sample. The Alegria® Test Strip holds a complete set of reagents. Included are enzyme conjugate, enzyme substrate, sample buffer and a test specific control. Two wells of the Alegria® Test Strip are coated with a 25-OH vitamin D_3/D_2 antibody and serve as reaction wells for one control and one patient sample. Two more wells of the Alegria® Test Strip are coated with a 25-OH vitamin D tracer or a 25-OH vitamin D control respectively.
The determination is based on a competitive enzyme linked immunosorbent assay (ELISA) with the following steps: The sample is pipetted into well No 1. Inside the Alegria® Random Access Analyser the sample is mixed with tracer reagent and the 25-OH vitamin D_3/D_2 is delivered from vitamin D binding protein. 25-OH vitamin D and tracer reagent coated in well No 2 are suspended with buffer. Sample and control are then transferred to the reaction wells No 3 and No 4 where 25-OH vitamin D_3/D_2 and 25-OH vitamin D tracer reagent compete for binding to the coated 25-OH vitamin D_3/D_2 antibody. Complexes are formed between 25-OH vitamin D_3/D_2 and antibody or 25-OH vitamin D tracer reagent and antibody. After incubation, a first washing step removes unbound and unspecifically bound molecules. Subsequently added enzyme conjugate binds to the immobilized tracer-antibody complexes. After incubation, a second washing step removes unbound enzyme conjugate. Addition of enzyme substrate solution results in hydrolisation and color development during incubation. The intensity of the blue color can be measured photometrically at 650 nm. The intensity of the blue color correlates inversely with the concentration of vitamin D in the sample.
The Alegria® Test Strip utilizes the proprietary SMC®-Technology (Sensotronic Memorized Calibration): information about the assay, analysis and evaluation, and the lot-specific expiry date is contained on the barcode printed on each Alegria® Test Strip.
The Alegria® Test Strip can be used with the diagnostic instrument Alegria® - a fully automated Random Access Analyser. By means of SMC®-Technology data encoded on the barcode are transferred from the Alegria® Test Strip to the instrument and the assay is automatically processed and evaluated. The instrument reads the date of expiry and rejects further processing if the Alegria® Test Strip is out of date.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- System fluid contains acid, classifiaction is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:

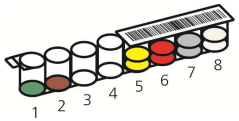
Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store Alegria® strips in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.

CONTENTS OF THE KIT

24	ORG 270	Sufficient for 24 determinations
ALEGRIA TEST STRIPS	24	**Alegria® Test Strips** are modules of 8 wells each composed of:



Well 1: green; coated with 25-OH Vitamin D tracer
Well 2: brown; coated with 25-OH Vitamin D tracer + 25-OH Vitamin D control
Wells 3 + 4: coated with antibody (reaction wells)
Well 5: Buffer: yellow.
Well 6: Enzyme Conjugate; light red; containing HRP conjugate; PBS, BSA, detergent, preservative ProClin 0.05%.
Well 7: Matrix; opaque; containing human serum matrix, Tris, BSA, preservative sodium azide 0.09%.
Well 8: TMB Substrate: clear; containing 3,3', 5,5'- Tetramethylbenzidin.
Antibodies detecting 25-OH Vitamin D_2 and 25-OH Vitamin D_3 are bound to reaction wells 3+4.

Code on barcode: ***VitD*** on printout: ***VitD3/D2***

WASH	1x 20 ml	**Wash Buffer**, containing Tris, detergent, preservative sodium azide 0.09%; 50 x concentrate
SYSTEM FLUID	1x 2.5 ml	**System Fluid**, contains acid; 1000 x concentrate
	1	**Certificate of Analysis**

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store Alegria® Test Strips sealed and dessicated in the clip bag provided.
- Shelf life of the unopended test kit is **12** months from day of production.
  Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and System Fluid are stable for at least 30 days when stored at 2-8°C.
  Once transferred to the reagent container we recommend consumption on the same day.

MATERIALS REQUIRED

- Vortex mixer
- Pipettes for **80** µl
- Measuring cylinder for 1000 ml and 2500 ml
- Distilled or deionized water

AUXILLIARY REAGENTS

ORG 271 25-OH Vitamin D_3/D_2 Control Set

25-OH Vitamin D_3/D_2 Control Set is an external quality control material for the quantitative Alegria® 25-OH Vitamin D_3/D_2 assay. This product is intended for professional in vitro diagnostic use only.
The Control Set contains Control A und Control B 2.5 ml each with a defined concentration of 25-OH Vitamin D_3/D_2 (see label) in Tris 0.7%, NaCl 0.8% with human serum matrix, BSA 0.5% and sodium azid 0,09% as preservative. Ready to use. For 30 Determinations. Store ORG 271 at 2-8°C in the dark.
Unopened controls are stabil until expiry date (see label). Once opened use up within 4 months.
Procedure in the Alegria® assay: Pipette **80** µl control at the bottom of well 1 of the Alegria test strip.
This Control Set is available separately.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Do not expose specimens to heat, sun, or strong light during storage and usage.
- Avoid repetitive freezing and thawing of serum or plasma samples.

- Testing of heat-inactivated sera is not recommended.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- All materials must be at room temperature (20-28°C) prior to use.
- To avoid carryover or contamination, change the pipette tip between samples.

PREPARATION OF REAGENTS

WASH

Dilute the content of the Wash Buffer concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use. Transfer the diluted Wash Buffer into the instrument reagent container. If only one Alegria run is to be performed on one day we recommend transferring only 500 ml diluted Wash Buffer.

SYSTEM FLUID

Dilute the content of the System Fluid concentrate (1000x) with distilled or deionised water to a final volume of 2500 ml prior to use. Transfer the diluted System Fluid into the instrument reagent container.

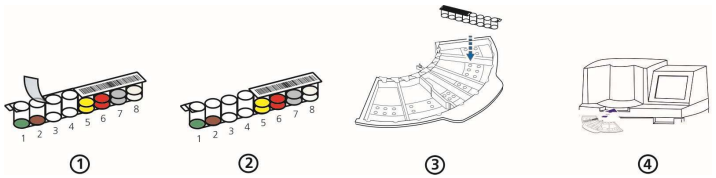
ALEGRIA TEST STRIPS

Take the required number of Alegria® Test Strips out of the clip bag and let them reach room temperature (20-28° C). Do not remove foil covering the empty wells until you are ready to start the assay.

TEST PROCEDURE

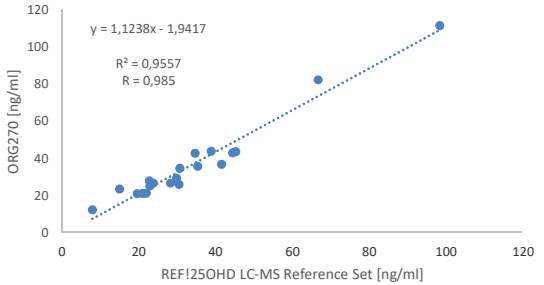
Alegria® Test Strips with SMC® technology are used with the diagnostic instrument Alegria®.
Detailed information about operating the instrument can be taken from the Instrument User Manual.

(1) Remove the foil from the empty wells 1 to 4 of the Alegria® Test Strip.
Do not remove foil with printed barcode, covering wells 5 to 8.
(2) Pipette **80** µl undiluted patient sample (serum or plasma) or ready to use control ORG 271 at the bottom of well 1.
(3) Insert the strip into the SysTray. (3) Insert the strip into the SysTray.
(4) Place loaded SysTrays into the correct position in the Alegria® instrument and start run. All further steps will be done automatically. The test run is completed when the instrument starts printing the results.
Caution: Alegria® Test Strips 25-OH Vitamin D_3/D_2 need a special performance and therefore they cannot be combined with other Alegria® tests in the same run.



CALIBRATION

The calibration is traceable to the ID-LC/Tandem MS reference preparation REF!25OHD (Labquality, IQAS).



CALCULATION OF RESULTS

By means of SMC® Technology (Sensotronic Memorized Calibration), all test data are transferred to the system through individual barcodes on the Alegria® Test Strip. Calculation and interpretation of results will be performed automatically.

PERFORMANCE CHARACTERISTICS

Measuring range

The calculation range of this Alegria® assay is 5 - 200 ng/ml

Interpretation of results

Suggested classification ranges of vitamin D status according to literature (Ref 5):
(Conversion factors: 1ng/ml = 2.5 nmol/l; 1 nmol/l = 0.4 ng/ml)

Deficiency:	< 12 ng/ml	(< 30 nmol/l)
Insufficiency:	12 - 20 ng/ml	(30 - 50 nmol/l)
Sufficiency:	> 20 - 150 ng/ml	(> 50 - 375 nmol/l)

Limitations of the Procedure

According to literature several reference ranges are suggested. Examples:
Ref 5
Deutsche Gesellschaft für Ernährung (DGE), (ÖGE), (SGE), (SVE), Referenzwerte für die Nährstoffzufuhr, Vitamin D. 2012; ISBN 978-3-86528-128-9).
Deficiency: <12 ng/ml; Insufficiency: 12-20 ng/ml; Sufficiency: >20-160 ng/ml; Hypervitaminosis 160-500 ng/ml
Ref 9
Holick, M.F. Vitamin D deficiency. N. Engl. J.M. 2007, 357: 266-281.
Deficiency: <20 ng/ml; Insufficiency: 21-29 ng/ml; Sufficiency: >30 ng/ml; Intoxication >150 ng/ml
Ref 16
National Osteoporosis Society 2013, Vitamin D and Bone Health: A Practical Clinical Guideline for Patient Management. Deficiency: <12 ng/ml; Insufficiency: 12-20 ng/ml; Sufficiency: >20 ng/ml

According to the literature, factors such as nutrition, season, skin color, age or culture affect the normal 25-OH vitamin D levels.
The concentration ranges for the classification of vitamin D supply should be considered a recommendation.
Each laboratory should establish its own ranges according to ISO 15189:2007 Requirements for quality and competence particular to medical laboratories or other applicable criteria.

Linearity

Samples containing high levels 25-OH vitamin D were serially diluted to demonstrate the dynamic range of the assay. 25-OH vitamin D concentration for each dilution was calculated by means of SMC® Technology.

Sample	Dilution	Observed	Expected	O/E
		[ng/ml]	[ng/ml]	%
1	1:1	170.2	200.0	85
	1:2	101.6	100.0	102
	1:4	44.6	50.0	89
	1:8	21.7	25.0	87
	1:16	12.9	12.5	104
2	1:1	129.2	120.0	107
	1:2	62.6	60.0	104
	1:4	25.1	30.0	84
	1:8	15.3	15.0	102
3	1:1	58.5	62.6	94
	1:2	28.5	31.3	91
	1:4	14.7	15.6	94
	1:8	9.7	7.8	124
	1:16	4.1	3.9	105

Detection limit

Smallest ammount of Vitamin D detectable 5 ng/ml

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.
Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below

Intra-Assay		
Sample	Mean	% CV
	[ng/ml]	%
1	16.3	10.7
2	50.1	6.1
3	107.5	3.9

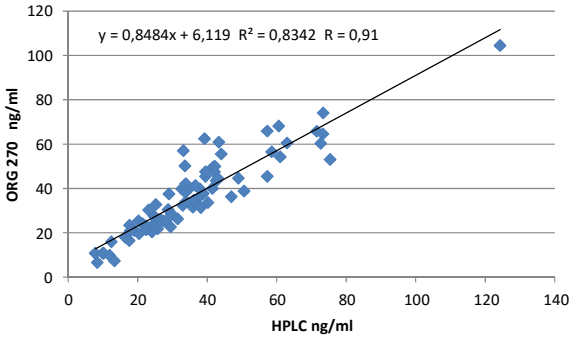
Inter-Assay		
Sample	Mean	% CV
	[ng/ml]	%
1	16.5	14.7
2	45.6	7.9
3	98.9	4.2

Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

In a comparative study 74 vitamin D serum samples from persons aged 8 to 89, 2/3 female and 1/3 male were analyzed. A high correlation was found between Alegria® 25-OH Vitamin D_3/D_2 and HPLC method:
R = 0.91



Specificity

Specificity was determined by measurement of cross-reactivity to 25-OH vitamin D related substances.
Cross-reactivity is stated in % relative to 25-OH Vitamin D_3 reactivity:

25-OH Vitamin D_3	100 %
25-OH Vitamin D_2	100.4 %
Vitamin D_3 (Cholecalciferol)	< 0.1 %

Vitamin D_2 (Ergocalciferol) < 0.6 %

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Notice to the user (European Union):
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control

Former version: *ORG 270_IFU_EN_QM130718_2016-01-21_4* Reason for revision: *Introduction electronic IFU on homepage*