VITAMIN D
QUANTI CARD

Fluorescence immunoassay (antigen-antibody) for quantitative measurement of Vitamin D (Total) in human serum

INTRODUCTION
Vitamin D is a steroid hormone involved in the intestinal aborption of calcium and the regulation of calcium homeostasis. The two major forms of vitamin D, vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol), have isomeric structure, but D2 is supposed to be less active than D3. Physiological vitamin D3 levels results not only from dietry uptake but can also be produced from a cholestrol precursor, in the skin during sun exposure. D2 is obtained from plant sources and only represents less than 5% of the total vitamin D of the body. In the liver, the vitamin D is hydroxylated to 25-hydroxyvitamin D (25-OH D), the major circulating metabolite of vitamin D. Vitamin D and 25-OH D enter the circulation bound to vitamin D binding protein (VDBP). It is widely accepted that the measurement of circulating 25-OH D provides better information with respect to patients vitamin D status and allows its use in diagnosis of hypovitaminosis. Determination of 25-OH D in serum will support the diagnois and therapy control of postmenopausal osteoporosis, rickets in children, osteomalacia, renal osteodystrophy, neonatal hypocalcemia and hyperparathyroidism.

Vitamin D intoxication mostly occurs during a large intake of pharmaceuticals preparations of vitamin D and may lead to hypercalcemia and nephrocalcinosis in susceptible infants.

INTENDED USE
Vitamin D Quanti Card is a sensitive immunoassay for the quantitative determination of Vitamin D (Total) in human serum with device reader.

PRINCIPLE (ANTIGEN-ANTIBODY REACTION)
Vitamin D Quanti Card is based on principle of competitive immunoassay. The vitamin D from the sample is first released/extracted. The assay is based on competition between extracted vitamin D from test sample and vitamin D biotin conjugate for the limited no of binding sites of specfific antibodies fluorochrome conjugate. As a result higher concentration of vitamin D produces a lower fluorescence signal and vice versa. The signal is interpreted and displayed on device reader in form of results.

MATERIALS PROVIDED
Vitamin D Quanti Card Test kit contains following components to perform the assay:
1. Vitamin D Quanti Card 2. Conjugate-A 3. Releasing Buffer
4. Instruction Manual

MATERIAL REQUIRED, BUT NOT PROVIDED
iQuant Analyzer
Micropipette & Microtips
Stop Watch
Dual iHeating Block

KIT PRESENTATION
10 Test Pack

STORAGE AND STABILITY
Vitamin D Quanti Card should be stored at 2-8ºC in the coolest & driest area available. Expiry date on the kit indicates the date beyond which kit should not be used. The kit should not be frozen & must be protected from exposure to humidity.

DESCRIPTION OF SYMBOLS USED
The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the British and European Standard EN ISO 15223-1:2016.

	Manufactured By	IVD	In vitro diagnostic medical device
Σ	No. of tests		See Instruction for use
LOT	Lot Number Batch Number	2°C 8°C	Temperature Limitation
	Manufacturing Date		Caution, see instruction for use
	Expiry Date	REF	Catalogue Number
	Do not use if package is damaged		Keep away from sunlight
	Single use only		

WARNING FOR USERS
CAUTION: ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for in vitro diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. All materials used in the assay and samples should be disposed off in accordance with established safety procedures and guidelines.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. Conjugate-A contains Sodium Azide as a preservative. If these material are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline "Safety Management No. CDC-22", Decontamination of Laboratory Sink Drains to remove Azide salts" (Centre for Disease Control, Atlanta, Georgia, April 30, 1976).
10. **Use only serum sample for testing.**

PRECAUTIONS
In order to obtain reproducible results, the following instructions must be followed:
1. Use disposable gloves while handling potentially infectious samples and performing the assay. Wash hands thoroughly afterwards.
2. Do not use the kit beyond the expiry date.
3. Do not mix reagents from different batches.
4. Do not open the foil pouch until it attains room temperature.
5. Do not re-use the test device.
6. Use separate pipette tips for each sample in order to avoid cross-contamination of samples which could cause erroneous results.
7. For best results, follow the given test procedure and storage instructions strictly.

Important Note: Vitamin-D Quanti Card is only operational in conjuction with iQuant Analyzer.

SAMPLE / SPECIMEN COLLECTION AND STORAGE
1. Only serum samples should be used with this test.
2. If serum specimens cannot be tested immediately, they should be refrigerated at 2-8ºC. For storage for more than 3 days, freeze the specimen at -20ºC or below.
3. Repeated freezing and thawing of the specimen should be avoided.
4. Specimens containing precipitate or particulate matter may yield inconsistent test results. Such specimens must be centrifuged and the clear supernatant should only be used for testing.
5. The use of hemolytic, lipaemic, icteric or bacterially contaminated specimens should be avoided as it may lead to erroneous results.

BEFORE YOU START
1. Switch on the iHeating block before starting the test procedure and make sure it should attain the factory preset (50°C) temperature required for testing. As the iHeating block is switched ON, its "RED" LED indicator will glow and upon attaining the desired temperature its "GREEN" LED will glow. It may take approximately 10-15 min to achieve the factory preset temperature. The "RED" LED indicator may go ON/OFF as per heating requirement. However "GREEN" LED will glow continuously. Once its "GREEN" LED is ON, the instrument is ready for use.
2. Plug in the iQuant analyzer. Press the Power button of the iQuant analyzer, it will take approximately 1 min for its self-checking and when the test screen will come, one can start the test procedure.
3. Plug in the iVortexer.
4. Bring the complete test kit and samples to be tested to room temperature (RT) prior to testing.

RT 20-30°C

TEST PROCEDURE
Step A: Vitamin D Releasing Step:

i) Label the microtubes with patient ID.

ii) Take **100µl** of **serum sample** in a fresh micro tube provided in the kit only and add **100µl** of **Releasing Buffer** followed by addition of **50µl of Conjugate A**. Mix well by **vortexing for 15 sec** using iVortexer and incubate for **25 minutes** by placing it in the pre-heated iHeating Block. **Use separate tips for each pipetting step.**

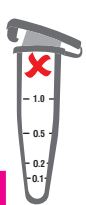


Fig. (a)

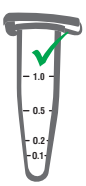
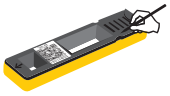


Fig. (b)

Note: Close the lid of the microtube properly by pressing it twice to ensure proper locking as shown in fig. (b) and it should be followed for each step.

Step B: Assay Procedure for running the test:



i) Remove the test cartridge from foil pouch prior to use and place it on a flat and dry surface. The test should be performed immediately after removing the test cartridge from foil pouch.

ii) Label the test cartridge with patient's name or identification number. **DO NOT write on QR Code**.

iii) After incubation is complete, load the **75µl** of the mixed solution to the sample well of the cartridge & insert the test cartridge into the cartridge slot of dual iheating block. **Care should be taken to avoid any spillage on the QR-Code pasted on the cartridge & on the result reading window.**

iv) Allow the reaction to occur **for 25 minutes** at room temperature (20-30°C). In the meantime enter the patient's details in the iQuant Analyzer testing window and select the Vitamin-D test from the pop down menu in the testing window of the i Quant analyzer.

v) Insert the test cartridge after **25** minutes into the iQuant Analyzer with arrow (←) marked side on the top of the cartridge facing towards the iQuant Analyzer.

vi) Press the "RUN" icon on the iQuant analyzer test window.

vii) Note down the Vitamin-D value displayed on the result window of the iQuant Analyzer.

viii) Discard the Vitamin D Quanti Card immediately after reading the results at 25 minute considering it to be potentially infectious.

MEASURING RANGE:

The measuring range of Vitamin-D Qunati card is 4-80 ng/ml. Samples showing results as >80ng/ml should be diluted to get the actual value of the sample. For calculation of the concentration this dilution factor shall be taken into account.

Detection Limit: 4 ng/ml

Conversion of ng/ml to nmol/L: nmol/L = 2.5 X ng/ml

INTERPRETATION OF RESULTS

Interpret the results as per table given below:

Deficient	:	<10 ng/ml
Insufficient	:	10 - 30 ng/ml
Sufficient	:	30-100 ng/ml
Intoxication	:	> 100 ng/ml

PERFORMANCE CHARACTERISTICS OF VITAMIN D QUANTI CARD

1. Conformity with NIST Standards

The results of Vitamin D Quanti Card are in conformity with NIST standards. Standard Reference Material (972a) containing level 1, 2, 3 & 4 for Vitamin D from National Institute of Standards & Technology (NIST), US Department of Commerce, USA. These standards are checked on Vitamin D Quanti Cards and results are tabulated as below:

NIST Standard Reference Material (SRM) for Vitamin D metabolites

Levels	Certified Values in NIST SRM 972a (ng/mL)	Value Observed in Vitamin D Quanti Card (ng/mL)	Co-relation
Level 1	28 + 1.1	29.2	Results with all 4 levels of standard are within defined range and found to be in co-relation.
Level 2	20.20 + 0.52	20.5	
Level 3	33.1 + 0.8	33.8	
Level 4	29.4 + 0.9	30.0	

2. Precision

Within-run and between-run precisions have been determined by testing 10 replicates of 3 control samples: low, medium & high. The C.V (%) of all the 3 control samples were within 10%.

3. Accuracy

The accuracy of Vitamin D Quanti Card was checked with 110 clinical specimen spanning the assay range. Vitamin D concentration of 110 samples were compared with LC-MS/MS and commercially available ELISA test kit. The assay demonstrated excellent corelation with results determined by LC-MS/MS and commercially available ELISA test kit. The accuracy parameters are as given below:

Regression Parameter	LC-MS/MS	Commercially Available ELISA Kit
Slope	0.9632	0.9742
Intercept	1.7112	1.6081
R^2 (Corelation Coefficient)	0.997	0.993

4. Specificity

There was no significant interference with the Vitamin D measurement when other biomolecules such as Bilirubin (40 mg/ml), Triglyceride (500 mg/dl), haemoglobin (500 mg/ml) were added to the test specimen with much higher level than in normal blood.

LIMITATIONS AND INTERFERENCES

1. The test procedure, precautions and interpretation of results for this test must be strictly followed.
2. The results obtained from the use of this kit should be used only as an adjunct to other diagnosttic procedure and information available to the physician.
3. Any modification to the above procedure and / or use of other reagents will invalidate the test procedure.
4. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an in vitro diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

REFERENCE:

1. Freeman J, Wilson K et al. influence of Vitamin D binding protein on accuracy of 25 Hydroxy Vitamin D measurement using the ADVIA untaur Vitamin D Total Assay.
2. Holick MF, Binkley NC et. Al. Evaluation, treatment and prevention of Vitamin D deficiency: an Endocrine Society Clinical Practice Guideline. J. Clin. Endocrinol Metabol 96 (2011) 1911-1930.
3. Yetley EA. Assessing the Vitamin D status of the US population. Am. J. Clin Nutr 88(2008) 558-564.

TROUBLE SHOOTING

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. Unexpected low test result	a) Incubation period of Releasing step less than 25 minutes.	Repeat the test with incubation period of 25 minutes.
	b) High volume of Conjugate-A used.	Use accurate volume of Conjugate-A.
	c) Reagents used were too cold & were not brought to room temperature (R.T.)	Bring the whole test kit to R.T. before testing.
2. Unexpected high test result	a) Low volume of reaction mixture applied to sample well of cartridge.	Repeat test with appropriate loading volume.
	b) Expired test kit used.	Repeat test using a new test kit that has not passed the expiration date.
	c) Low volume of Conjugate-A used.	Use accurate volume of Conjugate-A.
	d) Conjugate-A is continuously exposed to light for more than 8 hrs.	Always recap the Conjugate-A vial after use. Store at 2-8°C.
3. Test Result do not match with other Elisa or CLIA method	a) Result on Elisa or CLIA kit can be more or less than actual value due to improper calibration of instrument.	Recheck the sample after proper calibration of instrument.
	b) Procedural error.	Check the procedure and perform test again as per kit product insert. Retest the sample on standard ELISA or CLIA kit to confirm the correct value of the sample.

For in-vitro diagnostic use only, not for medicinal use

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