Fluoro-Check™ Vitamin D

For in vitro Diagnostic Use

One–step quantitative time resolved fluorescence immuno-chromatography assay for the detection of vitamin D (total 25(OH)D2/D3) in human serum and plasma

1. INTENDED USE

The Fluoro-Check™ Vitamin D test is a time resolved fluorescence immuno-chromatography for the quantitative determination of vitamin D (total 25(OH)D2/D3) in human serum and plasma (EDTA, lithium-heparin, and citrate). Measurements of total 25(OH)D2/D3 are used to aid in the assessment of vitamin D sufficiency. Test results should be interpreted by the physician along with other test results and patient clinical symptoms findings.

2. SUMMARY AND EXPLANATION OF THE TEST

Vitamin D is a fat-soluble prohormone and known for its role in regulating calcium and phosphorus levels in bone mineralization. Sunlight exposure produces vitamin D via photochemical conversion of 7-dehydrocholesterol in the epidermis and is the primary source of vitamin D. Seasonal changes, amount of exposure, sunscreen use, and skin pigmentation can cause variation in the amount of vitamin D produced in the body. The vitamin D can be also absorbed from food and vitamin supplements with an estimated 10-20% absorbed in this manner. In circulation, 25(OH) vitamin D is bound to vitamin D-binding protein or albumin at 1,000 times higher concentrations than the active form 1,25(OH) vitamin D. Additionally, the 25(OH) form has the half-life of 2-3 weeks and the less stable 1,25(OH)2 form has the half-life of a few hours. 25(OH) vitamin D exists as D2 (ergocalciferol) or D3 (cholecalciferol) isomers and both are available with supplements. Often, total 25(OH) vitamin D is measured to assess the sufficiency in a patient and make appropriate clinical decision.

3. PRINCIPLE

The Fluoro-Check™ Vitamin D test is a membrane-based immuno-chromatography assay for the evaluation of Vitamin D in patient blood stream. The membrane strip contains a test line printed with BSA-modified vitamin D and a control line printed with Chicken IgY antibody. A reaction tube and pretreatment solution is provided with the test. The reaction tube contains a lyophilized mixture of fluorescence particles coupled with vitamin D-specific antibody and fluorescence particles coupled with goat anti-chicken IgY antibody. The pretreatment tube contains a pretreatment solution that separates the Vitamin D molecule from the Vitamin D-binding proteins. The sample specimen and the pretreatment solution are transferred into the reaction tube and incubated in the Fluoro-Check™ Heating Block. During this process, the vitamin D molecules in sample specimen bind to the vitamin D-specific antibody coupled with fluorescence particles. This mixed solution is applied to the sample well of the test. If Vitamin D molecules are present in the sample, it competes with the BSA-modified vitamin D printed on the membrane for the fluorescence particles coupled with vitamin D-specific antibody resulting in a reverse signal. The goat anti-chicken IgY antibody coupled with fluorescence particles are captured by chicken IgY in the control line and this indicates assay validity. The test device should be read by Fluoro-Check™ TRF Reader to measure the concentration of Vitamin D. The analyzer quantifies fluorescence intensity of the test line and convert it to the concentration of the Vitamin D in the sample specimen.

4. REAGENT

The Fluoro-Check™ Vitamin D test contains all the reagents necessary for the determination of Vitamin D in human serum and plasma. The device contains a membrane strip coated with BSA-conjugated Vitamin D on the test line. Reaction tube contains Vitamin D-specific antibody coupled with fluorescence particles in a lyophilized form. The pretreatment tube contains a pretreatment solution to prepare the free Vitamin D in the sample specimen.

5. MATERIALS

Provided

- 20 Test devices sealed in pouch with desiccant
- 1 Tube rack pouch containing 20 reaction tubes and 1 pretreatment solution tube
- 1 QR Card for calibration (lot-specific)
- 1 Instruction for use

Required but not provided

- Serum or plasma collection container
- Positive and negative quality control materials
- Adjustable Micropipette (80uL) and microtips
- Timer
- Fluoro-Checker™ TRF Reader
- Fluoro-Check™ Heating Block

6. STORAGE AND STABILITY

The test device should be stored at 2°C ~ 30°C in the original sealed pouch for the duration of shelf life.

7. PRECAUTIONS

- For *in-vitro* diagnostic and professional use only.
- Do not use hemolyzed specimens because hemolysis affects test result.
- Handle all specimens as potentially infectious. Proper handling and disposal methods should be established.
- Do not interchange the components between different lots.
- To avoid cross contamination, use a new pipette tip for each clinical sample tested.
- Do not use test if the pouch is damaged or improperly sealed.
- Do not use reaction tube if the tube rack pouch is damaged or improperly sealed.
- Do not use all components beyond the expiration date.

8. SPECIMEN COLLECTION AND PREPARATION

- The blood samples should be collected under the standard laboratory conditions.
- Plasma samples with heparin, sodium citrate or EDTA as the anticoagulant can be used for testing with this product. Other blood anticoagulants have not been evaluated.
- For use of Serum samples: collect the blood in a tube without anticoagulant and allow clotting for at least 25 minutes before centrifugation.

  ***NOTE:** Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variation in these products may exist between manufacturers and, at times, production batches.

- Optimal results will be obtained when patient sample was tested immediately after collection. Serum or plasma samples may be refrigerated for 24 hours at 2°C ~ 8°C. If testing cannot be performed within 24 hours or in case of the shipment of samples, freeze them at -20°C or below.
- Refrigerated or frozen serum or plasma specimen should reach room temperature and be homogeneous prior to testing.

9. TEST PROCEDURE AND PROTOCOL

- Collect specimen according to instructions in “Specimen Collection and Preparation”.
- Test, reagents and sample should be brought to room temperature (20°C ~ 30°C) prior to testing.
- Set the heating block to 43°C and check the temperature prior to the start of testing. Fluoro-Check™ Heating Block is recommended for use with Fluoro-Check™ Vitamin D. The Fluoro-Check™ Heating Block is preset to 43°C.
- Remove the test from the sealed pouch immediately before use.
- Remove the reaction tube and pretreatment solution tube from the sealed pouch immediately before use and then close sealing line tightly.
- Transfer 80 µL of sample (serum or plasma) to reaction tube. A new pipette tip should be used for each sample application.
- Add 80 µL of pretreatment solution into the reaction tube.
- Mix the solution by inverting the reaction tube at least 20 times and insert it into Fluoro-Check™ Heating Block.
- Incubate reaction tube in Fluoro-Check™ Heating Block for 10 min.
- After incubation, mix the solution once more by inverting the reaction tube at least 20 times.
- Transfer 80 µL of the mixed solution in reaction tube into sample well of the test using a new tip and leave it for 15 min.
- Insert the test into the Fluoro-Check™ TRF Reader and run the test.
- Read the test result displayed on the Fluoro-Check™ TRF Reader. Use the Fluoro-Check™ TRF Reader according to the instruction manual.

10. INTERPRETATION OF RESULTS

The signal intensity of test line can be analyzed by Fluoro-Check™ TRF Reader and the results are expressed as the concentration of analyte using predetermined calibration curves specific for Fluoro-Check™ Vitamin D tests. For specimens with the vitamin D concentration of 9 ng/mL to 85 ng/mL, the test results will be displayed quantitatively.

11. LIMITATIONS

- The Fluoro-Check™ Vitamin D test is for professional and in vitro diagnostic use only.
- A definitive clinical diagnosis should not be made based on the results of a single test. Confirmation of test results should be made by a physician along with clinical symptoms and laboratory findings.
- Failure to follow the test procedure may adversely affect test performance and/or invalidate the test result.

12. QUALITY CONTROL

The presence of fluorescence band in the control area of the window acts as an internal control to ensure an adequate volume of sample has been added. In the absence of this control band, the associated test result is invalid and must be retested. Good laboratory practice recommends quality control to ensure proper test performance. Quality control materials are available from commercial sources and should be tested by following same procedures as the patient samples. Good laboratory practice suggests that external controls should be tested with every new lot or in case of questionable test result. If the quality control procedures in your laboratory require more frequent use of controls to verify the test results, follow your laboratory-specific procedures. The recommended requirement for testing the IQC provided with the instrument is for regular time period. When the test result is questionable for any reason, contact the customer support.

13. REFERENCE RANGE

The reference range of Fluoro-Check™ Vitamin D was determined in comparison with LC-MS/MS Total vitamin D (Agilent technologies). Reference range of LC-MS/MS is summarized in the table below and correlate well with Fluoro-Check™ Vitamin D. The reference range may be different if a quantitative assay other than LC-MS/MS Total vitamin D (Agilent technologies) is used.

Vitamin D (total 25(OH)D2/D3)	Status
< 10 ng/mL	Deficient
10 ng/mL ~ 30 ng/mL	Insufficient
30 ng/mL ~ 100 ng/mL	Sufficient

14. PERFORMANCE CHARACTERISTICS

1. Detection limits

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of quantification (LoQ) studies were performed according to CLSI guideline EP17-A. The LoB was determined by testing 60 replicates of vitamin D depleted serum sample. To determine LoD, six levels of relatively low vitamin D concentration samples (7.4 ng/mL ~ 9.7 ng/mL) were tested with 10 replicates per level. From this result, a tentative LoD was calculated and further verified by evaluating the additional 10 replicates per day for 5 days. The LoQ was determined to be the reliably detected concentration in consideration of acceptance criteria of precision.

Detection limit		Conc. (ng/mL)
Limit of Blank	(LoB)	4.7
Limit of Detection	(LoD)	8.4
Limit of Quantification	(LoQ)	8.4

2. Linearity

Linearity study of Fluoro-Check™ Vitamin D was performed according to CLSI guideline EP6-A. Data set was collected with samples covering dynamic range of Fluoro-Check™ Vitamin D assay and it was confirmed that the linear model was capable of interpolating the experimental points. Fluoro-Check™ Vitamin D was demonstrated to be linear from 9 ng/mL to 85 ng/mL.

3. Analytical Specificity (Cross-Reactivity)

Fluoro-Check™ Vitamin D demonstrated less than 10% of recovery difference with the following substances at the concentrations indicated below. The evaluation was performed based on CLSI guideline EP7-A2.

Substance	Conc. (ng/mL)
Vitamin D2 (ergocalciferol)	500
Vitamin D3 (cholecalciferol)	500
1,25-(OH)2-vitamin D2	100
1,25-(OH)2-vitamin D3	100
3-epimer-25-OH vitamin D3 (OHD3)	100
24,25-(OH)2-vitamin D3	25
Paricalcitol (Zemplar)	25

4. Interfering Substances

Fluoro-Check™ Vitamin D demonstrated less than 20% of recovery difference with the following substances at the concentrations indicated below. The evaluation was performed based on CLSI guideline EP7-A2.

Substance	Conc.
L-Ascorbic Acid	3 mg/dL
Biotin	6000 µg/mL
Hemoglobin	500 mg/dL
Rheumatoid Factor (RF)	536 IU/mL
Human Anti Mouse Antibody (HAMA)	1000 ng/mL
Bilirubin(conjugated)	30 mg/dL
Bilirubin(unconjugated)	30 mg/dL
Ibuprofen	30 mg/dL
Uric Acid	20 mg/dL
Acetamininophen	20 mg/dL
Triglycerides	1500 mg/dL

5. Precision Test

Precision of Fluoro-Check™ Vitamin D with Fluoro-Check™ TRF Reader was performed according to CLSI guideline EP5-A. Three levels of plasma sample were tested in 2 replicates, 2 runs per day for 20 days. The results are summarized in the table below

Analyte	Conc. (ng/mL)	Within Run		Total Run	
		SD	CV (%)	SD	CV (%)
Vitamin D	15	1.22	7.9%	1.22	7.9%
	30	2.57	8.2%	2.68	8.5%
	45	5.61	11.5%	5.61	11.5%

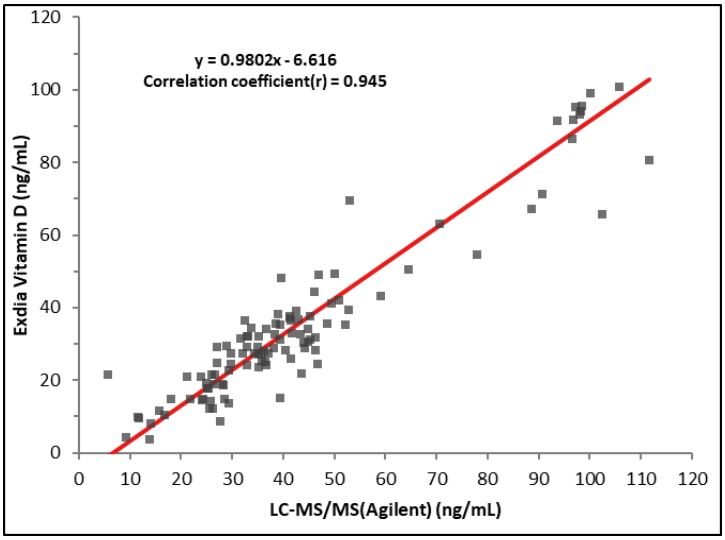
6. Matrix Comparison Study

Matrix comparison of Fluoro-Check™ Vitamin D with Fluoro-Check™ TRF Reader was performed according to CLSI guideline EP14-A2. Whole blood specimen was divided into 8 vials and spiked with different amount of vitamin D standard material to have the concentration of 10 ng/mL ~ 100 ng/mL. The prepared set of 8 vials was further treated with 4 types of tubes to generate the different matrices: Serum, heparinized plasma, sodium citrate treated plasma, EDTA treated plasma. Each sample was tested in 3 replicates and the results were statistically analyzed. The correlation analysis using Passing-Bablok fit was performed with reference to serum. The strong positive correlation between the tested matrices was observed as in the table below, which indicated that samples in these matrices can be tested equivalently on Fluoro-Check™ Vitamin D.

Sample types	Serum	Plasma-Heparin	Plasma-EDTA	Plasma-Sodium Citrate
Correlation coefficient	N/A	0.965 ($p < 0.0001$)	0.962 ($p < 0.0001$)	0.981 ($p < 0.0001$)
95% CI	N/A	0.920 to 0.985	0.912 to 0.984	0.956 0.992

7. Method Comparison Study

Method comparison of Fluoro-Check™ Vitamin D with Fluoro-Check™ TRF Reader was performed according to CLSI guideline EP09-A2. Total 107 plasma samples were evaluated on both Fluoro-Check™ Vitamin D and LC-MS/MS Total vitamin D (Agilent technologies). The results were analyzed by Passing-Bablok analysis and summarized in the figure and table below.



Analyte	Sample number	Concentration range (ng/mL)	Intercept	Slope	Correlation coefficient
Vitamin D	107	5.72 ~ 111.58	-6.616	0.9802	0.945

15. REFERENCES

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