

# Vitamin D Test Cassette with Fluorescent Immunoassay Expected to Improve Assay Efficiency

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## Abstract

Recent evidence of vitamin D effects on non-skeletal bones, coupled with the recognition that vitamin D deficiency is common, has brought renewed attention to this hormone. Vitamin D is produced by skin exposed to ultraviolet B radiation and can also be obtained from dietary sources, including supplements. People at high risk for vitamin D deficiency include those with inadequate sun exposure, limited oral intake, or impaired intestinal absorption. Vitamin D adequacy is best determined by measuring blood concentrations of 25-hydroxyvitamin D. Average daily vitamin D intake in the general population and current dietary reference intakes are often insufficient to maintain optimal vitamin D levels. Vitamin D is composed of two bioequivalent forms. Ergocalciferol, other name for vitamin D<sub>2</sub>, can be acquired through dietary sources of vegetables as well as oral supplementation. The primary sources of vitamin D<sub>3</sub> (cholecalciferol) were oral supplements, oily fish, fortified foods such as milk, fruit juices, margarine, yoghurt, cereals, and soy, and skin exposure to Ultraviolet B (UVB) radiation from sunlight. Apart from vitamin D-rich fish, most foods contain between 50 and 200 international units of vitamin D per serving. This value varies widely in different parts of the world because fortification can significantly increase the availability of vitamin D through the diet. Both D<sub>2</sub> and D<sub>3</sub> have no biological effects. After being absorbed from the intestine, they undergo liver metabolism to become 25(OH) vitamin D [25(OH)D], which is made up of 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>. 25(OH)D, also known as calcidiol, is then converted by the action of the enzyme 1-hydroxylase in the kidney and other tissues to 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D], also known as oestriol. The primary function of vitamin D is carried out by calcitriol, an endocrine and autocrine hormone that works by activating the vitamin D receptors in cells. Clinicians may recommend vitamin D supplementation but are unsure how to select the optimal dose and type, and how to use tests to monitor therapy. This review provides an overview of the complications of vitamin D deficiency and how to perform tests to detect vitamin D levels in the body to evaluate the performance characteristics of Fiatest Vitamin D Test Cassette.

**Keywords:** Vitamin D; 25-hydroxyvitamin; Fluorescent immunoassay ; Aerobic bacteria

## Introduction

The term "vitamin D" refers to a class of fat-soluble secosteroids that promotes the intestinal absorbance of calcium, iron, magnesium, phosphate, and zinc. The two most significant of these substances in the human body are vitamin D<sub>3</sub> and vitamin D<sub>2</sub> [1,2]. Unlike Vitamin D<sub>2</sub>, which is primarily derived through diet, Vitamin D<sub>3</sub> is naturally produced by the skin on exposure to UV radiation. The liver is where vitamin D is transferred and converted to 25-hydroxyvitamin D. The 25-hydroxyvitamin D blood test is used in medicine to measure the body's vitamin D levels. The most accurate measure of vitamin D status is considered to be the blood level of 25-hydroxyvitamin D (containing D<sub>2</sub> and D<sub>3</sub>). According to limited studies, vitamin D supplementation helped some hospitalized patients experience lower COVID-19 symptoms. Today, vitamin D deficient is viewed as a worldwide epidemic [3]. The majority of human body cells have vitamin D receptors, which means that for them to function properly, vitamin D levels must be adequate. Many of them previously believed, vitamin D deficiency poses serious health hazards. Osteoporosis, osteochondrosis, multiple sclerosis, cardiovascular disease, complications during pregnancy, diabetes, depression, stroke,

autoimmune diseases, influenza, cancer, infectious diseases, Alzheimer's disease, obesity, and high mortality are just a few of the serious diseases that are linked to vitamin deficiency [4]. Therefore, testing (25-OH) vitamin D levels is now considered a "medically necessary screening test" and maintaining adequate levels may improve not only bone health but overall health and well-being. [5] Vitamin D deficiency reduces the immune system activity in every individuals including the individuals effected with SARS-CoV.

## Materials and Methods

### The sunlight exposure and Vitamin D<sub>3</sub>

Since more than 500 million years ago, the planet has created vitamin D, a vitamin that is produced in sunlight. When exposed to sunlight, the skin's 7-dehydrocholesterol absorbs UVB rays and transforms into previtamin D<sub>3</sub>, which then isomerizes to vitamin D<sub>3</sub>. In addition to absorbing UVB rays, previtamin D<sub>3</sub> and vitamin D<sub>3</sub> are also transformed into a range of photoproducts, some of which have particular biological characteristics. Season, time of day, latitude,

altitudes, air pollution, skin pigmentation, sunscreen usage, walking on glass and plastic, and ageing all have a significant impact on sunlight-induced vitamin D production [1]. Exposure to sun in the early hours of the day improves the body activity in COVID-19 individuals reducing the respiratory distress.

### Different effects of vitamin D2 and D3 on the immune system

According to a recent study, vitamin D2 and D3 supplements showed differing impacts on immune system-related genes [6]. These results are important because the majority of earlier research has been unable to establish a clear distinction between the effects of vitamin D2 or D3 treatment.

The majority of studies that have been released to date have demonstrated that the impact on circulating vitamin D levels is the primary distinction between vitamin D2 and D3 administration. Studies have evidenced that vitamin D3 is superior at increasing the body's vitamin D levels. A recent analysis of the available data indicated that vitamin D3 administration increased vitamin D levels in the body more effectively than vitamin D2 administration. Not all studies, meanwhile, support this theory.

Few research studies states that vitamin D2 supplementation is more preferable than vitamin D3 supplementation. In a study, vitamin D2 performed better in treating immunological issues in people receiving steroid therapy. Apart from raising vitamin D levels in the body, there isn't much proof that vitamin D3 pills are superior to vitamin D2 supplements. According to study, vitamin D3 increased calcium levels more effectively when compared to vitamin D2.

### Test method for Vitamin D status

Vitamin D is quickly transformed to 25(OH)D when it is ingested or produced mostly by skin, but only a small portion of 25(OH)D is converted to 1,25(OH)2D in serum. As a result, measuring total 25(OH)D levels is a reliable test to determine the body's vitamin D stores. Vitamin D deficiency can be identified and tracked using total 25(OH)D levels, while treatment monitoring can be facilitated by quantitative 25(OH)D2 and 25(OH)D3 levels.

An absence of an increase in the corresponding 25(OH)D2 or 25(OH)D3 and total 25(OH)D levels, for particular, in patients who have not noticed clinical improvement after taking D2 or D3 supplements, may indicate inadequate dose, nonadherence, or malabsorption.

In individuals with healthy kidneys and bones, normal serum calcium and phosphorus levels are maintained primarily through the interaction of two hormones: Parathyroid Hormone (PTH) and ossified triol. In COVID-19 patients with vitamin D deficiency, secondary HPT causes the release of calcium that has been stored in the bones and causes the kidneys to reabsorb calcium to ensure normal serum calcium and phosphorus levels.

Vitamin D deficiency in COVID-19 individuals is usually accompanied by normal blood calcium and phosphorus levels, high normal or elevated levels of parathyroid hormone, normal to elevated levels of total alkaline phosphatase, low 24-hour urinary calcium excretion rates, and low levels of total 25(OH)D. Although this is the rare, patients with severe chronic vitamin D deficiency may experience considerable hypophosphatemia and/or hypocalcemia. By evaluating 1,25(OH)2D levels, doctors shouldn't be able to identify

vitamin D deficiency. As a result, patients with vitamin D deficiency who have elevated levels of metanephrine may have their vitamin D status misinterpreted. Osteotriol levels are typically normal or even elevated in these patients.

## Results and Discussion

### Evaluation of Fiatest vitamin D test cassette (serum/plasma)

**Principle of the Test:** The Vitamin D Test Cassette (Serum/Plasma) is based on a fluorescent immunoassay for the detection of vitamin D. The specimen sample moves from the sample pad to the absorbent pad. The vitamin D in the sample is competing with the VD-BSA antigen on the envelope. Less vitamin D is available in the sample the more fluorescent microspheres coupled to the anti-*vd* antibody are captured by the VD-BSA antigen encapsulated on the membrane (Test line). The concentration of vitamin D in the sample is inversely associated with the strength of the fluorescence signal captured on the T-line. Based on the test's fluorescence intensity and the standard curve, the fluorescence immunoassay analyzer may compute the sample's vitamin D concentration.

**Materials and directions for use:** Materials provided included a test cassette, specimen collection tubes with buffer, ID card, and a package insert. To complete this test, additional materials are required but not provided. They include a timer, centrifuge, a fluorescence immunoassay analyzer, pipettes and specimen collection containers. Prior to testing, can let test, specimen, buffer, and/or controls get to room temperature (15 °C-30 °C).

Before beginning the test, turn on the adapter's fluorescence immunoassay analyzer's power source. Select the test mode and specimen after taking out the ID card and inserting it into the appropriate slot. The test cassette should then be taken out of the foil pouch and used within an hour. The assay should be carried out as soon as the foil packet is opened for the most accurate results.

Pipette 25 µl of serum or plasma into the buffer tube while the test cassette is horizontal and spotless. After mixing the specimen and buffer thoroughly for 2-5 minutes, use a pipette to pour 75 µl of the diluted sample into the sample wells of the test card. Turn on the timer and the test results should be read by the fluorescence immunoassay analyzer after 15 minutes (Table 1). Each laboratory should determine the applicability of the reference range through experiments and establish its own reference value range if necessary to ensure that it can correctly reflect the situation of a particular population as vitamin D intake varies by region, age group and skin color [7,8].

Concentrations	Clinical Reference
< 10 ng/mL	Deficient
10-30 ng/mL	Insufficient
> 30 ng/mL	Sufficient

Table 1: Expected results.

## Conclusion

At Vitamin D is important for bone and non-bone health. It is now well established that many people have vitamin D levels that are below the currently recommended optimal health levels. Worldwide, vitamin D is obtained primarily through exposure of patients with

COVID-19 to UVB radiation and skin production of vitamin D from sunlight. Latitude, cultural dress habits, seasons, sun protection and sun exposure may all limit vitamin D production. The Fiatest Vitamin D Test Cassette uses a fluorescence immunoassay with an adapted fluorescence immunoassay analyzer to determine vitamin D level in the human body by reading test results 15 minutes after a simple operation is completed.

As described earlier, vitamin D is not actually a vitamin, but rather a hormone that promotes calcium absorption in the body. It has important health implications for both teenagers and children, as well as aged populations. People who don't get enough vitamin D will not only experience fatigue but also have their bones mature more slowly. The main cause is that low vitamin D levels make the body's mitochondria, which are where most of the body's cells' aerobic respiration occurs, less effective. Fortunately, vitamin D deficiency can be identified through testing and be treated under the guidance of a healthcare practitioner. Treatment options can vary for each individual, But in order to keep the body healthy, low vitamin D levels must be treated. A vitamin D pill can lessen the severity of COVID-19. In order to treat COVID-19, vitamin D should be suggested as an adjuvant therapy.

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