

Evaluation of the Performance Characteristics of the Vitamin D Rapid Test Cassette for Semi-Quantitative Self-Testing of 25-Hydroxyvitamin D in Human Fingertick Whole Blood

Lei Zhang^{1,*}, Feng Yang², Junzhe Zhu³

¹*Zhejiang Gongshang University, Hangzhou, Zhejiang, 310018, China*

²*Community Health Service Center, Hangzhou, Zhejiang, 310000, China*

³*Wenzhou Medical University, Wenzhou, Zhejiang, 310000, China*

Keywords: Vitamin D; Rapid Test Cassette; Chromatographic Immunoassay; 25-Hydroxyvitamin D; Point-Of-Care Testing; Self-Testing

Abstract: The Vitamin D Rapid Test Cassette is a rapid chromatographic immunoassay designed for semi-quantitative detection of 25-hydroxyvitamin D (25(OH)D) in human fingertick whole blood, with potential for self-testing by individuals. This study aimed to comprehensively evaluate its performance characteristics, including sensitivity, specificity and accuracy, through in-house clinical studies. A total of 90 specimens [4 vitamin D-deficient (1-10ng/ml), 56 insufficient (10-30ng/ml), and 30 sufficient (30-100ng/ml)] from individuals with suspected deficiency or insufficiency were tested. Results showed a relative sensitivity of 95%, specificity of 93.3% and accuracy of 94.4%. The limit of detection covered the three critical ranges (10ng/mL, 10-30ng/ml, 30-100ng/ml), and intra-assay/inter-assay precision was satisfactory (>95% correct identification). These findings indicate the cassette is a reliable, rapid, and practical tool for initial vitamin D status screening, including self-testing scenarios, aiding timely decisions on supplementation and health management.

1. Introduction

Vitamin D plays a crucial role in maintaining bone health, as it facilitates calcium absorption in the intestine and helps regulate calcium and phosphate metabolism^[1]. Additionally, emerging evidence suggests that vitamin D may be involved in various other physiological processes, including immune function modulation, cell cycle regulation and cardiovascular health^[2]. Vitamin D deficiency or insufficiency is a global health issue, affecting a significant proportion of the population, especially in regions with limited sunlight exposure, certain ethnic groups and individuals with specific lifestyle or dietary habits^[3].

The accurate assessment of vitamin D status is essential for the diagnosis of deficiency or insufficiency and for guiding appropriate treatment, which typically involves vitamin D supplementation. The gold-standard method for measuring 25(OH)D, the major circulating form of vitamin D and the best indicator of vitamin D status, is liquid chromatography-tandem mass

spectrometry (LC-MS/MS)^[4]. However, LC-MS/MS requires expensive equipment, highly trained personnel and a well-equipped laboratory, which limits its use in point-of-care (POC) settings and in resource-limited areas.

Chromatographic immunoassays (LFIAs) offer simplicity, rapidity and portability, making them ideal for POC and potential self-testing. The Vitamin D Rapid Test Cassette, a novel LFIA, enables semi-quantitative 25(OH)D detection in fingerstick blood, addressing gaps in accessible testing. This study evaluates its performance against LC-MS/MS, focusing on suitability for self-testing, and discusses clinical utility.

2. Materials and Methods

2.1 Specimen Collection

A total of 90 specimens were collected from individuals with suspected vitamin D deficiency or insufficiency across multiple clinical sites, including general hospitals, clinics and health check-up centers. The specimens consisted of 4 Vitamin D deficient whole blood specimens (1-10ng/ml), 56 Vitamin D insufficient whole blood specimens (10-30ng/ml) and 30 Vitamin D sufficient whole blood specimens (30-100ng/ml). All specimens were collected following standard procedures.

If not tested immediately, whole blood specimens should be stored in a suitable environment. After thawing, they should be kept at 2 to 6 °C for a maximum of five days and must not exceed the original storage period. They should also not be repeatedly frozen. All specimens were first analyzed using the gold-standard LC-MS/MS method to determine their true vitamin D status (deficient: 25(OH)D < 20 ng/mL; insufficient: 20-30 ng/mL; sufficient: >30 ng/mL), and then tested with the Vitamin D Rapid Test Cassette.

2.2 Test Kit and Procedure

The Vitamin D Rapid Test is an immunoassay based on the principle of competitive binding. During testing, the mixture migrates upward on the membrane chromatographically by capillary action. The membrane is pre-coated with 25 (OH) D antigens on the test line region of the strip. During testing, 25 (OH) D present in the specimen will compete with 25 (OH) D on the test line for limited amount of anti-25 OH Vitamin D antibodies in the conjugate. The higher concentration of 25 (OH) D in the specimen, the lighter would be the T line. The result will be read according to the Color card provided with the kit. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

The clinical specimen was balanced at room temperature (15-30°C). The test cassette was placed on a clean and level experimental desk and approximately 20µL of fingerstick whole blood specimens were transferred to the "S" region of the test cassette. Two drops of buffer were then added to the "B" region and a timer was started. The results were read at 10 minutes, with a note that interpretation should not occur after 20 minutes.

Result interpretation: Two colored lines may appear during the test: one in the control region (C) and another in the test region (T). If the intensity of the line in the test region (T) matches the Deficient line (0-10 ng/mL) on the color card, it indicates a deficient Vitamin D level. If the line corresponds to the Insufficient line (10-30 ng/mL), it indicates an insufficient level. A faint line in the test region (T) equal to the Sufficient line (30-100 ng/mL) suggests sufficient Vitamin D levels. If only the control line appears and no line is present in the test region (T), it may indicate excess Vitamin D and consultation with a physician is recommended. If the control line fails to appear, it is likely due to incorrect procedural techniques; the procedure should be reviewed and the test

repeated with a new kit. If problems persist, the test kit should be discontinued and the local distributor contacted.

3. Results and Discussion

3.1 Results

3.1.1 Sensitivity and Specificity

The sensitivity of Vitamin D Rapid Test Cassette (OVD-402H) was 95.0%, the specificity was 93.3% and the relative accuracy of Vitamin D Rapid Test Cassette (OVD-402H) was 94.4% according to the 56 Vitamin D insufficient fingerstick whole blood specimens, 30 Vitamin D sufficient fingerstick whole blood and 4 Vitamin D deficient whole blood specimens.

3.1.2 Cross-reactivity and Interference

To evaluate cross-reactivity, the test cassette was challenged with various substances that may be present in human specimens and could potentially interfere with the test results. These included common hormones such as cortisol, thyroid hormones (T3, T4, TSH) and sex hormones (estrogen, testosterone), as well as drugs commonly used in clinical practice, such as statins, antihypertensive drugs and antibiotics. The test was also challenged with other forms of vitamin D, such as vitamin D₁ and vitamin D₃ metabolites other than 25(OH)D.

For interference studies, specimens were spiked with substances that may be present in abnormal amounts in certain patient populations, such as high levels of bilirubin, hemoglobin, and triglycerides. The test cassette was then used to analyze these spiked specimens and the results were compared with those of unspiked specimens to determine if there was any interference.

3.1.3 Precision

Intra-assay precision was evaluated by testing 10 replicates of low, medium and high 25(OH)D-containing specimens on the same day using the same test kit. The results showed that the correct identification rate was >95% for all specimen types and concentration levels. Inter-assay precision was evaluated by testing the same set of specimens on three different days using three different lots of test kits. The correct identification rate was also >95%, indicating good inter-assay precision.

3.2 Discussion

3.2.1 Performance Characteristics

The Vitamin D Rapid Test Cassette demonstrated relatively high sensitivity and specificity for the detection of vitamin D deficiency, insufficiency and sufficiency in whole blood specimens. The sensitivity for detecting deficiency and insufficiency was in the range of 95%, which is comparable to or better than some previously reported LFIA-based vitamin D tests^[5]. The high specificity (93.3%) ensures that the test has a low false-positive rate, reducing the likelihood of unnecessary follow-up tests or inappropriate treatment.

The ability to semi-quantitatively estimate 25(OH)D levels based on the intensity of the test line provides additional information compared to simple qualitative tests, which can be useful for monitoring the response to vitamin D supplementation over time.

3.2.2 Limitations

Despite its good performance, the Vitamin D Rapid Test Cassette has several limitations. First, like all LFIA, its sensitivity is lower than that of the gold-standard LC-MS/MS method, especially at the borderline between deficiency and insufficiency or in cases where the vitamin D levels are only slightly abnormal. False-negative results may occur and in cases where there is a high clinical suspicion of vitamin D deficiency or insufficiency but a negative result on the rapid test, confirmatory testing with LC-MS/MS may be necessary.

Second, the semi-quantitative nature of the test may not provide as accurate an estimate of 25(OH)D levels as quantitative methods. This may limit its use in situations where precise quantification is required, such as in research studies or in the management of patients with complex medical conditions where tight control of vitamin D levels is crucial.

Third, the test performance may be affected by improper specimen collection, handling or storage. For example, if whole blood specimens are not collected correctly or are exposed to excessive heat or humidity during storage, it may lead to inaccurate results. Healthcare providers need to be properly trained in specimen collection and handling procedures to ensure reliable test results.

Finally, the interference by high levels of RF, although observed in a small number of cases, is a concern. In patients with autoimmune diseases or other conditions associated with high RF levels, the test results should be interpreted with caution and additional confirmatory testing may be needed.

3.2.3 Comparison with Other Diagnostic Methods

Compared to other POC vitamin D tests, such as immuno-chromatographic strip tests that are only qualitative, the Vitamin D Rapid Test Cassette offers the advantage of semi-quantitative results. In comparison to LC-MS/MS, the rapid test is much faster (results available in 15 minutes compared to hours or days for LC-MS/MS), more convenient (no need for complex equipment or trained technicians) and more cost-effective, making it suitable for widespread screening in primary care, community health centers and self-testing. However, for accurate quantification and in cases where a high level of sensitivity is required, LC-MS/MS remains the gold-standard method.

4. Conclusion

The Vitamin D Rapid Test Cassette is a reliable and efficient chromatographic immunoassay for the semi-quantitative detection of 25(OH)D in whole blood specimens. It demonstrates relatively high sensitivity, specificity and precision, with comparable performance across the specimen. The test's rapid turnaround time, ease of use and semi-quantitative capabilities make it a valuable tool for the initial screening of vitamin D status, which can aid in timely clinical decision-making regarding vitamin D supplementation and management of related health conditions.

While the test has limitations, including lower sensitivity compared to LC-MS/MS and potential issues with specimen handling and interference, its strengths make it a practical option for POC testing in various healthcare settings. Future studies should focus on further improving the test's sensitivity and accuracy, evaluating its performance in different patient populations and exploring ways to reduce the impact of interfering substances.

References

- [1] Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357(3):266-281.
- [2] Pilz S, März W, Wellnitz B, et al. Vitamin D and mortality in older men. *J Clin Endocrinol Metab*. 2008; 93(7):

2603-2608.

[3] Looker AC, Johnson CL, Lacher DA, et al. Vitamin D status: United States, 2001-2006. *NCHS Data Brief*. 2011;(59): 1-8.

[4] Jones G. Assays for vitamin D and its metabolites. *Clin Biochem Rev*. 2008;29(Suppl 1):S63-S71.

[5] Wang Y, Zhang M, Li X, et al. Evaluation of a lateral flow immunoassay for rapid detection of 25-hydroxyvitamin D in human serum. *Clin Chim Acta*. 2019;495:240-245.