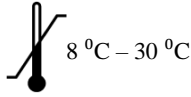




Rapid one step Quantitative Detection of 25-OH Vitamin D
in human blood/serum



Cat # **DG-2105-25**

INTENDED USE

Test4D™ is a rapid chromatographic immunoassay for the semi-quantitative detection of Vitamin D levels in human blood/serum.

This assay provides only a preliminary screening test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. The performance characteristics of this assay have not been established in a pediatric population.

PRINCIPLE

Test4D™ is based on the principle of a competitive immunoassay. The assay is based on the competition for 25-OH Vitamin D present in blood/serum sample and Vitamin D present on the test line for fixed number of antibody-gold conjugate. Depending upon the concentration of Vitamin D in blood/serum, there will be varying number of free antibody-gold conjugate molecules that will bind to Vitamin D on the test strip and will show a colored line in test line zone. A control line is present in the test window to work as procedural control.

MATERIALS PROVIDED

Each package contains:

1. 25 Test4D individually packed test devices in foil pouches.
2. 25 Lancets
3. 1 Buffer Vial containing Chase Buffer (5 mL) with stabilizers and thimerosal.
4. 1 Package Insert.

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer or clock
- Micro-pipette
- Plastic tube to mix blood/serum sample

SPECIMEN PREPARATION

1. Mix the blood/serum sample thoroughly by gently inverting the tube.
2. Use 10 µl of fresh blood sample or 5 µl of serum sample (Repeated freeze thaw of serum sample is not recommended. Bring frozen serum sample to room temperature before use.)
3. Assay must be performed immediately.

PROCEDURE

1. Remove the cassette from sealed pouch and place it on a hard flat surface with the view window facing up (use the cassette as soon as possible).
2. Using a micropipette, add 10 µl of blood or 5 µl of serum sample directly into the rectangular specimen well (A) of the cassette.

3. Add 3 full drops (or 100 µl by pipette drop wise) of Chase Buffer into the square buffer well (B) of the cassette (Do not move the cassette after addition of buffer).
4. Let cassette sit for 10 minutes and read your results immediately by reader as shown below. Results may change after the 10 minute mark.

TEST RESULTS AND REPORT

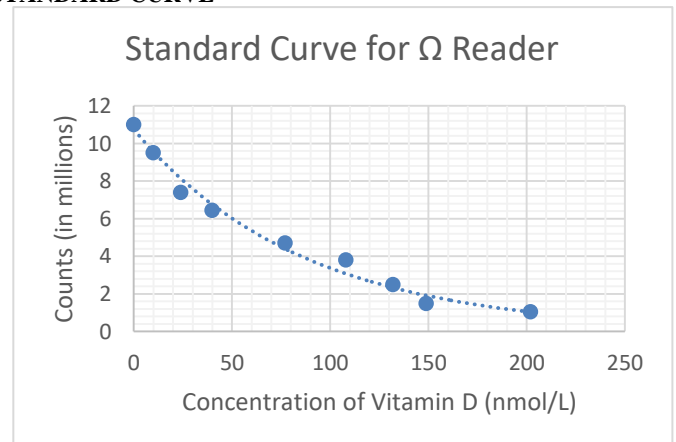
1. Place the cassette in the proper adapter.
2. Follow the instructions for scanning the cassette in opTrilyzer reader (See last page).
3. The results will be displayed on the screen in nmol/L. Note the results.



PRECAUTIONS

1. For in vitro diagnostic use only.
2. The test is designed for use with human blood/serum only.
3. Reagents and device must be at room temperature (8 °C – 30 °C).
4. Do not use if test device packaging is open or damaged.
5. Do not use the product beyond expiration date.
6. Handle all specimens as potentially infectious. Proper handling and disposal methods should be used according to good laboratory practices.
7. Read test results at 10 minutes as required. Results may deteriorate and may not be consistent after 10 minutes.
8. In order to avoid variable results mix the blood sample thoroughly.
9. A serum sample subjected to repeated freeze thaw cycle or a cloudy sample may give variable results.

STANDARD CURVE



PERFORMANCE CHARACTERISTICS:

Sensitivity: The sensitivity was determined based on 20 measurements of individual Vitamin D- free samples, calculated by subtracting 3 times of standard deviation from the mean. The sensitivity is 10 nmol/L.

Detection Range: The Detection range of Test4D™ is from 10 nmol/L to 207 nmol/L.

Spiking Studies: Low Vitamin D sample was spiked with Vitamin D to final concentrations of 24, 46, 72 and 145 nmol/L and estimated with Test4D™.

| Expected concentration (nmol/L) | Observed concentration (nmol/L) | Percentage Recovery |
|---------------------------------|---------------------------------|---------------------|
| 145.0 | 150.2 | 103.6 |
| 72.0 | 71.2 | 98.9 |
| 46.0 | 51.7 | 112.4 |
| 24.0 | 29.3 | 122.1 |

Percentage recovery was between 99 and 122% at the concentrations tested.

Accuracy: The accuracy of test was determined using 25 serum samples in comparison with LC-MS assay. The comparison result showed a correlation of 91%.

The accuracy was also evaluated using blood samples in comparison with corresponding serum samples. The comparison result showed a correlation of 94.8%.

| Blood (nmol/L) | Serum (nmol/L) |
|----------------|----------------|
| 48.1 | 42.4 |
| 78.4 | 84.4 |
| 89.5 | 86.2 |
| 93.4 | 105.6 |
| 109.9 | 132.6 |

Precision:

Precision measurements were conducted to evaluate repeatability. Precision studies were conducted using the samples that had a wide range of the assay. Variation was found to be less than 13% CV at all three levels tested.

| Sample | Replicates | Mean nmol/L | Coefficient of Variation(CV) |
|---------|------------|-------------|------------------------------|
| Serum 1 | 10 | 29.0 | 13.1% |
| Serum 2 | 10 | 72.9 | 8.9% |
| Serum 3 | 10 | 101.6 | 6.1% |

The inter-assay variation was calculated as 8.7 % by assaying the test samples over a period of eight days, and intra assay variation was found to be 6.2% by assaying 10 replicates on the same day.

Specificity:

No interference and cross reactivity was observed with added high concentrations of Vitamin A, bilirubin, triglycerides and cholesterol.

STORAGE AND STABILITY

The test device should be stored at 8 °C – 30 °C and will be stable until the expiration date stated on the package. The product is humidity sensitive and should be used immediately after being open.

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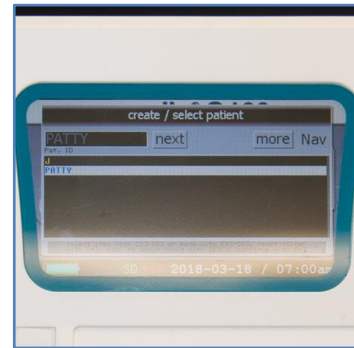
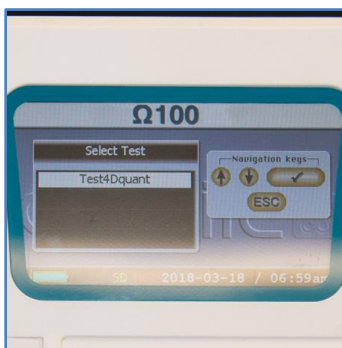
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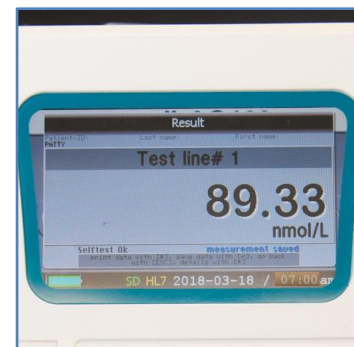
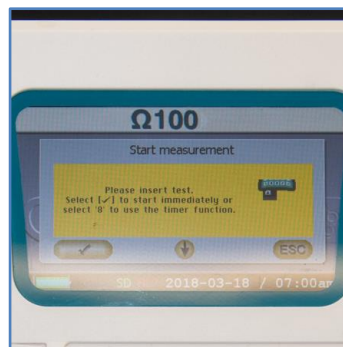
INSTRUCTIONS FOR USING oPTrilyzer (CE) WITH Test4D™



1. After the test is completed turn the Ω reader on by pressing the black button on the rear of the reader as shown in the picture above.
2. After a brief period of initializing and self-testing the reader will show the welcome screen with TEST menu highlighted.
3. Put the cassette in the proper adapter in the proper orientation. Put the adapter with the cassette inside the reader. Push the adapter inside the adapter slot until it fits properly.



4. Press the Select (✓) button and select the lot specific program from the list.
5. In the next screen, press the Select (✓) button to enter the id of a new patient or select an old patient from the list.



6. In the next screen, the operator needs to be selected from the list, or a new operator id can be entered. Press the Select (✓) button.
7. The reader will confirm if the test cassette has been inserted in the yellow screen. Press the Select (✓) button again to start scanning the cassette.
8. The concentration of Vitamin D in the sample will be displayed in nmol/L.