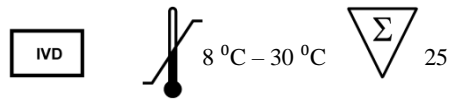


Rapid one step Quantitative Detection of 25-OH Vitamin D
in human blood/serum



INTENDED USE

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

This assay provides only a preliminary screening test result. A more specific alternative 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 molecule 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:

- 25 Test4D individually packed test devices in foil pouches.
- 25 Lancets
- 1 Buffer Vial containing Chase Buffer (5.0 mL) with stabilizers and thimerosal.
- 1 Package Insert
- RFID card

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer or clock
- Micro-pipette
- Cube reader

SPECIMEN PREPARATION

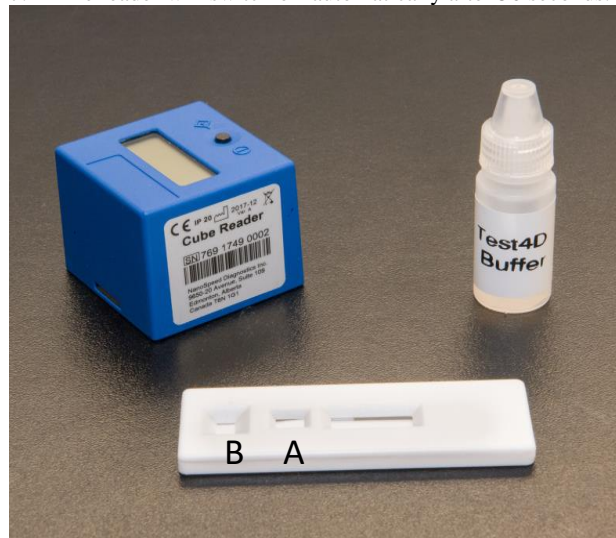
- Mix the blood/serum sample thoroughly by gently inverting the tube.
- Use 10 µl of fresh blood sample or 5 µl of serum sample (Repeated freeze thaw of serum sample is not recommended. Bring frozen serum sample to room temperature before use)
- Assay must be performed immediately.

PROCEDURE

- 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).
- Using a micropipette, add 10 µl of blood or 5 µl of serum sample directly into the rectangular specimen well (A) of the cassette.
- Add 3 full drops (or 100 µl with pipette drop wise) of Chase Buffer into the square buffer well (B) of the cassette (Do not move the cassette after addition of buffer).
- Let cassette sit for 10 minutes and immediately read your results by Cube reader as shown below. Results may change after the 10 minute mark.

TEST RESULTS AND REPORT

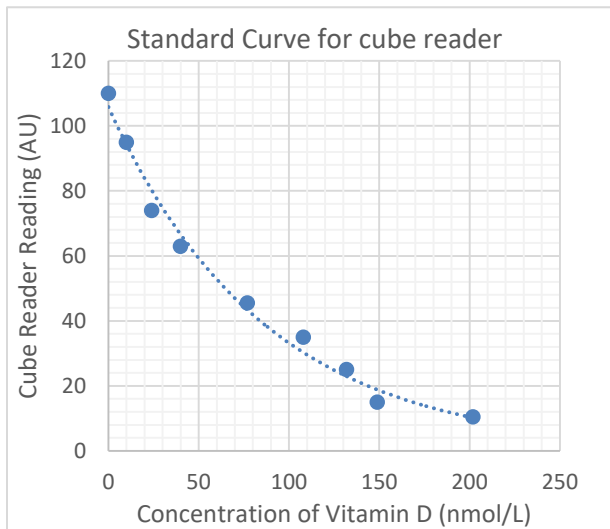
- Place the adapter on top of the test device properly. Place the cube reader on top of the adapter correctly.
- Press the black button on reader, it will display ON.
- Press the button again and display will read "RFID", Place the Lot Specific RFID card provided on the cube reader with each lot to upload the calibration data.
- Following a beep signal, "TEST" is displayed.
- Press the black button, it will display RUN.
- The results will scroll horizontally on the display screen in nmol/L. Note the results.
- The reader will switch off automatically after 50 seconds.



PRECAUTIONS

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

Standard curve using Cube Reader



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 10nmol/L to 207 nmol/L.

Spiking Studies: Low Vitamin D sample was spiked with Vitamin D to final concentrations of 12, 46, 71 and 140 nmol/L and estimated with Test4D™.

Expected concentration (nmol/L)	Observed concentration (nmol/L)	Percentage Recovery
140.0	154.9	110.6
71.0	69.4	97.7
46.0	53.0	115.2
12.0	11.5	95.8

Percentage recovery was between 96 and 115% 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 coefficient of 93.5%.

The accuracy was also evaluated using blood samples in comparison with corresponding serum samples. The comparison result showed a correlation coefficient of 96.6%:

Blood (nmol/L)	Serum (nmol/L)
43.2	35.6
61.6	67.6
83.2	89.8
87.6	94.6
93.4	83.2
113.8	123.6

Precision:

Precision measurements were conducted to evaluate repeatability. Precision studies were conducted using the samples that had a wide range of the assay. Precision was found to be less than 10% CV at all the three levels tested.

Sample	Replicates	Mean (nmol/L)	Coefficient of Variation (% CV)
Serum 1	10	14.3	6.4%
Serum 2	10	74.2	8.8%
Serum 3	10	100.8	8.3%

The inter-assay variation was calculated as 9.0 % by assaying the test samples over a period of eight days, and intra assay variation was found to be 7.2% 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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INSTRUCTIONS FOR USING CUBE READER (CE) WITH Test4D™



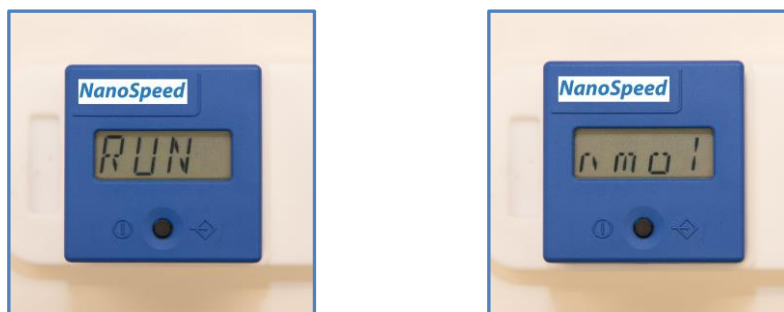
1. After the test is completed, put the Test4D™ cassette in the correct orientation into the adapter.
2. Put the Cube reader on the adapter so that it fits on the square space properly.



3. Turn on the cube reader by pressing the black button. After a brief period of self-testing, during which the reader shows WAIT, the reader will show ON. Press the black button.



4. The reader will show RFID. Place the Lot Specific RFID card on top of the Cube Reader. An audible signal will indicate successful reading of the RFID card. (For each lot of test4D, RFID card needs to be used only once. The reader stores the lot specific data from the first use. For all tests of the same lot the black button can be pressed to skip the use of RFID card).
5. The Cube Reader will show TEST. At this point, press the black button again.



6. The reader will display RUN and the Vitamin D concentration in the sample will be shown in nmol/L or ng/mL as per the setting. The result will scroll horizontally in the display.
7. The Cube Reader will shut down automatically in 50 seconds.