

## References

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# Serum 25-Hydroxyvitamin

## D<sub>2</sub> & D<sub>3</sub>

Information for Users



 CPA Accredited Laboratory



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## Sending Specimens for Analysis

**Sample requirement: minimum of 0.35mL of serum or plasma.** Please contact the laboratory if you would like to send a smaller volume of sample.

- Samples should be stored at 4°C prior to dispatch. There is no need to protect samples from light e.g. wrap in foil
- Send samples by first class post at ambient temperature to the address on the back of this leaflet

## Reference Ranges

| Total 25-Hydroxyvitamin D | (nmol/L)  |
|---------------------------|-----------|
| Severe Deficiency         | <15       |
| Deficiency                | 15 - 30   |
| Insufficiency             | 30.1-50   |
| Adequate Status           | 50.1-220  |
| High to toxic =           | 220.1-500 |
| Toxic                     | >500      |

***NB** to convert µg/L to nmol/L multiply by 2.5*

We also report the individual values for 25-hydroxyvitamin D<sub>2</sub> & D<sub>3</sub> in nmol/L for clinicians to monitor supplementation.

## Analytical Method

Our assay uses a liquid-liquid extraction method and tandem mass spectrometry (Waters TQD and Acquity UPLC) to measure 25-hydroxyvitamin D<sub>2</sub> and D<sub>3</sub>. Deuterated internal standards are employed for both 25-hydroxyvitamin D<sub>2</sub> and D<sub>3</sub>. Only 150uL of serum/plasma is required, however please contact the laboratory if you have very low volume samples that you would like to measure.

The limit of quantification for the assay (CV less than 15%), is 7.5 nmol/L for D<sub>3</sub> and 2.8 nmol/L for D<sub>2</sub>.

## Clinical Use

There is increasing evidence to show vitamin D deficiency is widespread in the UK. In a local study we found for our multi-ethnic inner city population (Birmingham) the prevalence of vitamin D deficiency, defined as a 25-hydroxyvitamin D concentration <25 nmol/L, was high at 24%, with 1 in 8 Caucasians, 1 in 4 Black Afro-Caribbean's and 1 in 3 Asians found to be deficient. Levels of deficiency were much higher in Asian women with almost 1 in 2 individuals (43%) found to have a vitamin D level below 25nmol/L.

Vitamin supplementation of food and

tablets comes in the form of both vitamin D<sub>2</sub> and Vitamin D<sub>3</sub>. Recent evidence has now shown that vitamin D<sub>2</sub> potency is less than one third that of vitamin D<sub>3</sub>, and its duration of action is much lower.

This is most likely due to a difference in affinity for vitamin-D binding globulin, with D<sub>2</sub> having a lower affinity, and subsequently a shorter circulating half-life. As a consequence, patients treated with D<sub>2</sub> may respond slower than expected to treatment. Therefore it is clinically important especially for monitoring patients treated for vitamin D deficiency (rather than just screening) both 25-hydroxyvitamin D<sub>2</sub> and D<sub>3</sub> are measured. It also allows clinicians to check patient compliance.

