

## Supplemental Methods: Calculation of bioavailable- and free 25-hydroxyvitamin D levels

[D<sub>total</sub>] = total measured vitamin D concentration (either 25(OH)D or 1,25(OH)<sub>2</sub>D)

[Alb] = measured albumin concentration

[DBP] = measured vitamin D binding protein concentration

[D<sub>Alb</sub>] = concentration of albumin-bound vitamin D

[D<sub>DBP</sub>] = concentration of DBP-bound vitamin D

[D<sub>free</sub>] = concentration of free (unbound) D

[D<sub>bioavailable</sub>] = concentration of Bioavailable D = [D<sub>free</sub>] + [D<sub>Alb</sub>]

K<sub>alb</sub> = affinity constant between vitamin D and albumin = 6 x 10<sup>5</sup> M<sup>-1</sup> (for 25-OH D) or 5.4 x 10<sup>4</sup> M<sup>-1</sup> (for 1,25-OH<sub>2</sub> D)

K<sub>DBP</sub> = affinity constant between vitamin D and DBP = 7 x 10<sup>8</sup> M<sup>-1</sup> (for 25-OH D) or 3.7 x 10<sup>7</sup> M<sup>-1</sup> (for 1,25-OH<sub>2</sub> D)

$$a = K_{DBP} \cdot K_{alb} \cdot [Alb] + K_{DBP}$$

$$b = K_{DBP} \cdot [DBP] - K_{DBP} \cdot [D_{total}] + K_{alb} \cdot [Alb] + 1$$

$$c = -[D_{total}]$$

$$[D_{free}] = [-b + \sqrt{b^2 - 4ac}] \div 2a$$

$$[D_{bioavailable}] = [D_{free}] + [D_{Alb}] = (K_{alb} \cdot [Alb] + 1) \cdot [D_{free}]$$