Quantitative Analysis of 25-Hydroxyvitamins D2 and D3 in Dried Blood Spot by Liquid Chromatography / Tandem Mass Spectrometry

Mark Newman[1], Theodore Brandon[1], Margaret Groves[1] and David Zava[1]

[1]ZRT Laboratory, Beaverton, OR, United States

Background

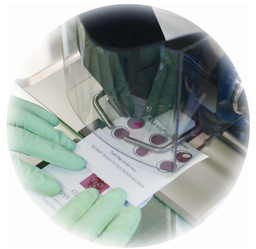
Hypovitaminosis D in children, even at non-rachitic levels, has been identified as a major contributor to the development of type I diabetes. Cod liver oil or vitamin D supplementation in infants is becoming a primary prevention strategy against type I diabetes in northern Europe, and maternal vitamin D supplementation is an alternative for breastfed infants. Both vitamin D2, derived from plants, and vitamin D3, naturally occurring in cod liver oil and produced in human skin from sunlight exposure, are used therapeutically. They are metabolized to 25-hydroxy D2 (25-OH D2) and 25-hydroxy D3 (25-OH D3) respectively, the precursors to the highly active 1,25$(OH)_2$ forms, and carried in the blood to target tissues. It is therefore important to monitor both 25-OH D2 and 25-OH D3 levels to determine vitamin D status.

Objective: A liquid chromatography/tandem mass spectrometry (LC-MS/MS) method was used to determine 25-OH D2 and 25-OH D3 levels in serum and blood spots, to assess the utility of a blood spot assay to assess neonatal vitamin D status during routine neonatal screening.

Methodology

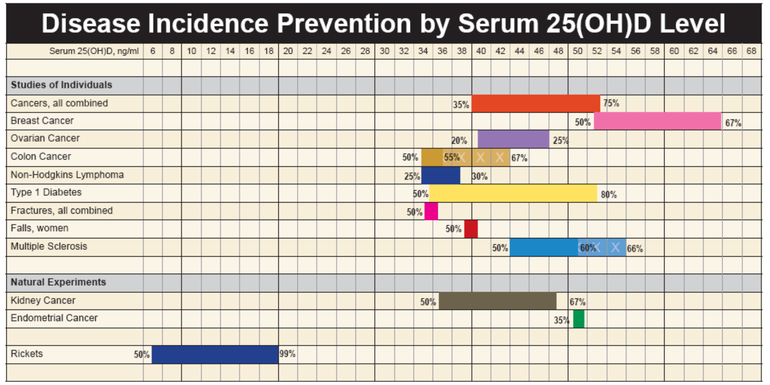
Design/Methods: Serum and blood spots were collected simultaneously from healthy individuals. Blood spots, obtained by finger stick or venous draw using anticoagulant, were dropped onto filter paper and allowed to dry. 6 mm spots were punched from each sample and extracted using an aqueous buffer followed by liquid hexane. After evaporation of the hexane and reconstitution in 50% methanol, 20 µL was injected into an LC-MS/MS system for measurement of 25-OH D2 and 25-OH D3. Serum levels were assessed using a validated method involving liquid hexane extraction following protein precipitation.

Convenient Sample Collection



- **Just a few drops of blood are needed from a heel- or finger-stick**
- **Full drops are applied to a filter paper card and allowed to dry (30-60 minutes)**
- **Samples can be mailed to the laboratory at the convenience of the physician/patient/researcher for analysis (turn-around time 2-5 days)**
- **Ideal for international shipment as blood spots do not require biohazard precautions**

Disease Incidence Prevention by Serum 25(OH)D Level



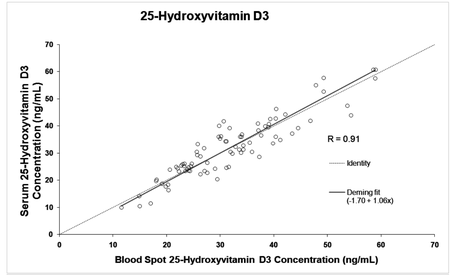
Data obtained from the following sources:

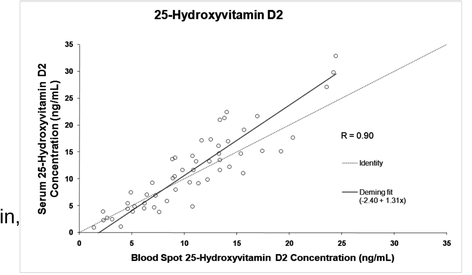
Chart Prepared by Garland, CF

Blood Spot and Serum Correlation

Validation of Blood Spot Assay

- **Precise: CVs <10% throughout the reference range**
- **Sensitive: Detection limit of 2ng/mL with just one drop of blood**
- **Linear results: From 3.5 – 200ng/mL**
- **Stable: Blood spots stable at room temperature for months**
- **Serum Correlation: Excellent (See Graphs)**





Other Blood Spot Tests at ZRT

- Thyroid Hormones and Antibodies
- Cardiometabolic Markers (HbA1c, Insulin, Triglycerides, hs-CRP)
- Steroid Hormones (Estradiol, DHEA-S, Progesterone, Testosterone, Cortisol)

Conclusions

The blood spot assay using LC-MS/MS is a reliable and convenient method to assess vitamin D status in neonates and children. Using mass spectrometry allows for quantitative analysis of both 25-hydroxyvitamin D2 and D3.

References

1. Hollis, B. Editorial: The Determination of Circulating 25-hydroxyvitamin D: no easy task. *J Clin Endocrinol Metab* 2004; **89**, 3149-3151. .
2. Maunsell, Z; Wright, D; Rainbow, S. Routine Isotope-Dilution Liquid Chromatography-Tandem Mass Spectrometry Assay for Simultaneous Measurement of the 25-Hydroxy Metabolites of Vitamins D2 and D3. *Clin Chem* 2005; **51:9**, 1683-1690.