LIAISON®

1,25 Dihydroxyvitamin D

First fully automated, extraction free immunoassay for the accurate detection of 1,25 Dihydroxyvitamin D
Synthesis of Vitamin D
Physiology of 1,25(OH)2 vitamin D
Calcium and phosphate homeostasis (CKD / FGF-23)
Bone Remodeling cycle
Metabolic Bone disorders (Drug Therapy)
Clinical background of 1,25(OH)2 vitamin D
Challenges in 1,25(OH)2 vitamin D measurement
Novel 1,25(OH)2 Assay format
BAP (Bone Alkaline Phosphatase) Assay
Advantage of Bone turnover marker
Vitamin D assay in Reproductive system
Synthesis of Vitamin D

7-dehydrocholesterol in epidermis (SPF ≥ 8, clothes, glass) 

Previtamin D₃ 

UVB (290-315 nm) 

Major Source: Sun 

Thermal heat from skin 

Vitamin D₃ (Cholecalciferol) 

Minor Source: Dietary 
Vitamin D₂ (ergocalciferol): Plants/supplements 
Vitamin D₃ (Cholecalciferol): 
Fish (cod liver oil), meat, fortified milk, egg yolk, butter 

Parathyroid hormone (+) 

25-hydroxylase 

25-hydroxyvitamin D₃ 25(OH)D₃ 

1-hydroxylase 

1,25-dihydroxyvitamin D₃ 

↑Calcium absorption (small intestine) 
↑Urinary calcium reabsorption (kidney) 
↑Bone mineralization
• skin, food, liver, parathyroid gland, kidney, bone, and small intestine all play a role

• The major form of Vitamin D, 25 (OH) Vitamin D (Calcidiol), has a limited biological activity.

• 1,25(OH)2 Vitamin D (Calcitriol) is a biologically active form

• 1,25 (OH)2 vitamin D controls calcium homeostasis in body by targeting intestines and bones
Physiology of 1,25(OH)\textsubscript{2} D

**Targeting:**

**Intestine:**
increase absorption of calcium and phosphate from the intestine

**Bone:**
increase bone resorption of calcium and phosphate

**Regulation:**
recall PTH functions to increase serum calcium, but decrease serum phosphate
Physiology of 1,25(OH)₂ D

1,25(OH)₂ D regulates PTH secretion from the parathyroid gland through negative feedback control.
Parathyroid hormone (PTH)
• released by low plasma calcium
• stimulates bone resorption
  (PTH receptor is on the osteoblasts which secretes IL-1 to activated osteoclasts)
• prevents calcium excretion by kidneys.
• stimulates calcitriol synthesis.

1,25-(OH)2-Vit. D (Calcitriol)
• stimulates bone resorption -> bone formation
• stimulates intestinal calcium absorption.
Physiology of PTH

- Phosphate Reabsorption
  - $1\alpha$ - hydroxylation of 25-OH vitamin D
- Bone Remodelling
  - Bone Resorption $>$ Bone Formation
- No direct effect
  - $Ca^{2+}$ absorption because of increased 1,25-(OH)$_2$ vitamin D
Phosphate homeostasis

Parathyroid hormone (PTH)

- inhibits phosphate reabsorption in proximal tubular cell

Fibroblast growth factor 23 (FGF23)

- polypeptide, synthesized by the osteoblasts
- involved in the calcification of bone matrix
- acts on the kidney
- decreases serum inorganic phosphate by inhibiting renal phosphate reabsorption and 1,25(OH)2 D (calcitriol) production

Summary of Phosphate Homeostasis Response to an Increase in Serum Phosphate

- PTH
- FGF-23
- Bone Resorption
- Pi reabsorption
- Pi absorption
- 1,25(OH)2 vitamin D
- u-phos
- sPi

* FGF-23, fibroblast growth factor 23
## Chronic Kidney Disease (CKD)

### Markers and Effect

<table>
<thead>
<tr>
<th>Markers</th>
<th>Effect</th>
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<tbody>
<tr>
<td>PTH</td>
<td>✓ decrease phosphate reabsorption</td>
</tr>
<tr>
<td></td>
<td>✓ increase calcium reabsorption</td>
</tr>
<tr>
<td>1,25 (OH)₂</td>
<td>✓ increase intestinal absorption of calcium and phosphate</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>✓ suppress PTH production</td>
</tr>
<tr>
<td>FGF-23</td>
<td>✓ facilitate excretion of phosphate</td>
</tr>
<tr>
<td></td>
<td>✓ promote calcitriol deficiency</td>
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Bone resorption begins when **RANKL** on the Osteoblasts membrane activates the **RANK** protein on the cell membrane of the Pre-Osteoblasts.
Bone resorption and bone formation are not separated, not independently regulated process.

**Bone Resorption**

Bone resorption begins when osteoclasts remove a portion of the bone to be replaced later by the action of osteoblasts. This is a vital step for signaling bone formation.

**Osteoclasts**

Bone resorption

**Osteoblasts**

Bone Formation

Osteoblasts lay down collagen and mineral deposits over the area previously remodeled by osteoclasts. Osteoblast activity is vital for maintaining bone mineral density and bone strength.
Metabolic bone disorders

- Normal bone
  - bone resorption or degradation is balanced by bone formation

- **Osteoporosis** (low bone mass and abnormal bone microarchitecture)
  - the rate of resorption exceeds the rate of formation

  **Causes:**
  - high bone turnover / endocrine disorder (primary and secondary hyperparathyroidism)
  - osteomalacia / renal failure / gastrointestinal disease (malabsorption syndrome)
  - long-term corticosteroid therapy / multiple myeloma / cancer metastatic to the bones

- **Paget’s disease** (a condition of abnormal bone formation)

  **Causes:**
  - excessive rates of bone remodeling, results in local lesions of abnormal bone matrix which results in fractures or neurological involvement.
Metabolic bone disorders

- **Rickets** (a condition that results in weak or soft bones in children)
  - the most common cause is vitamin D deficiency
  - **VDDR I:**
    - a deficiency of the renal 25-hydroxyvitamin D (25(OH)D)-1 alpha-hydroxylase.
  - **VDDR II:**
    - a spectrum of intracellular vitamin D receptor (VDR) defects

低磷血症性佝偻病（X连锁低磷血症性佝偻病）

- **Hypophosphatemic rickets** (X-linked hypophosphatemic rickets)
  - a form of rickets that is characterized by low serum phosphate levels and resistance to treatment with ultraviolet radiation or vitamin D ingestion
  - circulating FGF-23 concentrations have been shown to be 5 times higher in XLH patients, resulting in significant phosphaturia.
Disorders caused by drug therapies:

- immunosuppressive drugs for treating cancer and organ transplants
- heparin, used in kidney dialysis
- phenytoin (Dilantin.) for epilepsy
  (phenobarbital / rifampicin which induce hepatic P450 enzyme to accelerate the catabolism of Vitamin D)
- glucocorticoids (corticosteroids) for rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and asthma
- aluminium-containing antacids
Drug therapies for metabolic bone diseases

<table>
<thead>
<tr>
<th>Anti-resorptive agents:</th>
<th>Formation stimulating agents:</th>
<th>Agents inhibiting resorption and stimulating formation:</th>
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<tbody>
<tr>
<td>Estrogen (hormone replacement)</td>
<td>Sodium fluoride</td>
<td>Strontium ranelate</td>
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<tr>
<td>Phytoestrogen (hormone replacement)</td>
<td>Parathyroid hormone (human recombinant PTH (1-34))</td>
<td>Inhibits bone resorption</td>
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<tr>
<td>Calcium</td>
<td>Growth factors</td>
<td>PTH initially stimulates bone formation and later increases bone remodeling; increases spinal BMD. Suggested for treatment of patients with persistent osteoporosis after prior alendronate treatment. (Teriparatide (Forteo))</td>
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<tr>
<td>Selective estrogen receptor modulators</td>
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<td>Growth hormone therapy is used (and FDA approved) in the treatment of hypo-pituitarism and somatotropin deficiency in children and adults.</td>
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- **Bisphosphonates**
  - Prevent bone loss and increase BMD
  - (Alendronate (Fosamax); Risedronate (Actonel)).
  - Rigid administration is a disadvantage.

- **Calcitonin**
  - Treatment of osteoporosis and Paget’s disease, considered not as effective as bisphosphonates.
  - Decreased tolerance with long-term use.

- **Vitamin D**
  - Active form of vitamin D given to post-menopausal women who have osteoporosis in the spine.
1,25 (OH)$_2$D is the **active form** of Vitamin D, its production is tightly regulated through concentration of serum calcium, phosphorus and PTH.

- Low levels can be found in CKD, Vit D dependant rickets type 1, hypophosphatemic rickets, hypoparathyroidism

- High levels in Vit D dependant rickets type 2, Sarcoidosis, RA, IBD, primary hyperparathyroidism
Until now, all assays required a long, manual, operator dependent pre-analytical step due to the following facts:

- **The molecule circulates in low amounts**
  The blood levels of 1,25(OH)2 D being 100 to 1000 less than 25 OH D. (pg/mL concentration vs ng/mL concentrations)

- **Similarity with its metabolic precursor, 25-OH Vitamin D**
Novel Assay format

Concentrations of 1,25(OH)₂D are normally about 1000-fold lower than the precursor compound 25(OH)D.

Recombinant Fusion Protein (RFP)

Specific murine monoclonal antibody (MAB) which only recognizes the RFP Complex.

RFP changes conformation after capturing 1,25(OH)₂D and forms the RFP Complex.

RFP Complex is selectively recognized by the MAB.
Katharina Spanaus* and Arnold von Eckardstein

Evaluation of two fully automated immunoassay based tests for the measurement of 1α,25-dihydroxyvitamin D in human serum and comparison with LC-MS/MS

DOI 10.1515/cclm-2016-1074
Received November 25, 2016; accepted January 10, 2017
The DiaSorin test is very precise: total imprecision between 3.1 and 5.2%.

DiaSorin test measured 1,25(OH)₂ VitD with high accuracy.

The DiaSorin measurement results showed stronger correlations with the LC-MS/MS results ($r = 0.852$ vs. $r = 0.967$).

Nearly complete cross-reactivity with 1,25(OH)₂ Vit.D₂.

Due to its high sensitivity, low imprecision, broad measurement range, and good agreement with LC-MS/MS, the DiaSorin test is a valuable analytical option for the determination of 1,25(OH)₂ Vit.D.
LIAISON® XL 1,25 Dihydroxyvitamin D – Benefits

- First fully automated, extraction free
- First result in just 65 minutes
- Low sample volume (75 μL)
- More test from the same patient tube (eg 25-OH Vitamin D, PTH)
Bone and Mineral panel

The LIAISON® Bone & Mineral panel also includes:

<table>
<thead>
<tr>
<th>Test</th>
<th>Code</th>
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<tbody>
<tr>
<td>LIAISON® 25 OH Vitamin D TOTAL Assay</td>
<td>310600</td>
</tr>
<tr>
<td>LIAISON® 1-84 PTH</td>
<td>310630</td>
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<tr>
<td>LIAISON® N-TACT® PTH Gen II</td>
<td>317910</td>
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<tr>
<td>LIAISON® BAP OSTASE®</td>
<td>310970</td>
</tr>
<tr>
<td>LIAISON® Osteocalcin</td>
<td>310950</td>
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Bone-specific alkaline phosphatase (BAP), a glycoprotein that is found on the surface of osteoblasts.

- Reflects the biosynthetic activity of these bone-forming cell.

- Has shown to be a sensitive and reliable indicator of bone metabolism.
• Increased serum levels of BAP:
  (in conditions characterized by excessive bone turnover)
  - postmenopausal women/ osteoporosis/ Paget’s disease/ thyrotoxicosis / hyperparathyroidism / metastatic cancer, and are associated with rapid bone loss

• BAP levels decrease following anti-resorptive therapy in a dose-dependent manner.

• BAP identifies rapid bone losers, and accurately monitors the efficacy of hormone replacement-, bisphosphonate-, PTH analogue- and growth hormone-therapies
• To rapidly identify therapy responders and non-responders (detectable and significant changes in bone mineral density (BMD) take 18 to 24 months to develop, bone turnover marker takes 3-6 months after starting anti-resorptive therapy)

• To assess therapy efficacy and to determine the optimal therapy and dose of treatment.

• Biochemical bone marker reflect the whole-body rates of bone turnover, the combined measurement of bone marker and BMD provides more information on overall bone loss than BMD measurement at specific skeletal sites alone.
The cellular effect of vitamin D is mediated through the intra-nuclear vitamin D receptor (VDR).
Pandemic of Vitamin D deficiency

Vitamin D deficiency related diseases:

• Rickets in Children (兒童佝僂病)
• Osteoporosis, Osteomalacia (骨質疏鬆症)
• Cancer
• Type II Diabetes (第II型糖尿病)
• Cardiovascular disease (心血管疾病)
• Auto Immune Diseases (自體免疫疾病)
• Parkinson’s disease (帕金森氏病)
• Reproductive system (生殖系統)
Vitamin D and Reproductive system

• In the last few years, many researchers have studied the association of Vitamin D and reproductive health but there is still no single consensus on its influence in reproductive health.

• While it is a general observation that optimal level of Vitamin D is essential in PCOS, Endometriosis, Male infertility and IVF technique.

• but there has been no significant correlation between Vitamin D level and ovulation stimulation or embryo development.
Vitamin D and Female Reproduction

PCOS
(Polycystic ovary syndrome)

• Inverse association of serum Vit D and circulating androgens and insulin resistance in women with PCOS.

• Vit D supplementation improves menstrual frequency and metabolic syndromes.

PCOS: Hyper-androgenism / hirsutism / ovulatory and menstrual irregularities / insulin resistance / low pregnancy success rate / obesity / elevated cardiovascular disease risk
Endometriosis

- Higher 25(OH)D levels in women with endometriosis than control.

  - extrarenal site of Vit D synthesis and action: endometrial tissue
  - similar VDR polymorphism genotype
Male infertility

• Vitamin D metabolism enzymes (CYP24A1) are described in the human testis, the ejaculatory tract, mature spermatozoa and in the Leydig cells.

• Observed significantly reduced CYP24A1-expressing spermatozoa in the subfertile man compared with the healthy group. (P<0.001)

• Man with Vit D deficiency displayed a lower percentage of motile and morphologically normal sperm compared with Vit D sufficient subjects.
Vitamin D and Female Reproduction

IVF
(In Vitro Fertilization)

- High 25(OH)D levels are associated with higher clinical pregnancy rate.
- No significant difference.
- High follicular fluid 25(OH)D levels: lower clinical pregnancy rate.
AMH

• Premenopausal women were divided into 3 groups: age < 35, age 35-39, age > 40. For the youngest women AMH was negatively correlated with Vit D, whereas for the oldest women the relationship was reversed. The mean age at which the relationship was reversed was 35.

• AMH levels exhibited seasonal variation in women, with an 18% decrease in AMH levels in winter compared with summer. Vit D prevented seasonal AMH change. Vit D may be a positive regulator of AMH production in adults.
Large studies including all ethnic and racial groups would be required to proclaim the role of Vitamin D in infertility.
Thank you for your attention