1. INTENDED USE

The kit has been designed for the quantitative determination of 25-OH Vitamin D in human serum. The method can be used for samples over the range of 3-150 ng/ml. The test has to be performed on the Maglumi fully auto analyzer (including Maglumi 1000, Maglumi 2000, Maglumi 2000 plus).

2. SUMMARY AND EXPLANATION OF THE TEST

Vitamin D is a group of fat-soluble secosteroids. In humans, vitamin D is unique both because it functions as a prohormone and because the body can synthesize it (as vitamin D₃) when sun exposure is adequate (hence its nickname, the “sunshine vitamin”). Several forms (vitarins) of vitamin D exist (see table). The two major forms are vitamin D₃ or ergocalciferol, and vitamin D₂ or cholecalciferol. Vitamin D without a subscript refers to either D₂ or D₃ or both. These are known collectively as calciferol. Vitamin D₂ was chemically characterized in 1932. In 1936, the chemical structure of vitamin D₂ was established and resulted from the ultraviolet irradiation of 7-dehydrocholesterol.

Vitamin D₂ is a derivative of ergosterol, a membrane sterol named for the ergot fungus, which is produced by some organisms of phytoplankton, invertebrates, and fungi. The vitamin ergocalciferol (D₂) is produced in these organisms from ergosterol in response to UV irradiation. D₂ is not produced by land plants or vertebrates, because they lack the precursor ergosterol. The biological fate for producing 25(OH)D from vitamin D₂ is expected to be the same as for D₃.

Low blood calcidiol (25-hydroxy-vitamin D) can result from avoiding the sun. Deficiency results in impaired bone mineralization, and leads to bone softening diseases including: We often use dose of 5000IU / month to 50000IU / week of vitamin D₃ (or D₂) to treat the Vitamin D deficiency, fortified foods and nutritional supplements may contain some form of VD, in order to ensure accurate assessment of the total content of vitamin D. Vitamin D must be including all forms of vitamin D₃, D₂ and metabolites measured.

Recent studies have confirmed that children’s serum under 1 year old may exist 25-OH vitamin D in non-active 3 – epimer form, the test kit should be one of the important properties for the detection of only active ingredients, such as 25-OH vitamin D D₃ and D₂, but not inactive 3 – epimer of interference.

3. PRINCIPLE OF THE TEST

Competitive immunoluminometric assay:

Use a purified 25-OH Vitamin D antigen to label ABEI, and use 25-OH Vitamin D monoclonal antibody to label FITC. Sample, Calibrator, or Control, Displacing reagent, FITC Label and magnetic microbeads coated with anti-FITC are mixed thoroughly and incubated at 37 °C, forming antibody-antigen complexes; After sediment in a magnetic field, decant the supernatant, then cycle washing for the 2nd time, sample antigen and ABEI labeled antigen compete to combine with FITC labeled monoclonal antibody, forming antibody-antigen complexes. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of 25-OH Vitamin D present in controls or samples.

4. KIT COMPONENTS

4.1 Material supplies

<table>
<thead>
<tr>
<th>Reagent Integral for 100 determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nano magnetic microbeads: TRIS buffer, 1.2%(W/V), 0.2%NaNO₃, coated with sheep anti- FITC polyclonal antibody.</td>
</tr>
<tr>
<td>Calibrator low (lyophilized powder)</td>
</tr>
<tr>
<td>Calibrator high (lyophilized powder)</td>
</tr>
<tr>
<td>Displacing reagent: acidic buffer</td>
</tr>
<tr>
<td>ABEI label: 25-OH Vitamin D antigen labeled ABEI, contains BSA, 0.2%NaNO₃.</td>
</tr>
<tr>
<td>FITC label: 25-OH Vitamin D monoclonal antibody labeled FITC, contains BSA, 0.2%NaNO₃.</td>
</tr>
</tbody>
</table>

All reagents are provided ready-to-use.

The calibrators need to be dissolved with 3 ml distilled water before usage.

Accessories required but not provided

Maglumi Reaction module
Maglumi Starter kit 1+2
Maglumi Light check
Maglumi Wash /System Liquid
4.2 Preparation of the Reagent Integral

Before the sealing is removed, gentle and careful horizontal shaking of the Reagent Integral is essential (avoid foam formation!). Remove the sealing and turn the small wheel of the microbead compartment to and fro, until the colour of the suspension has changed into brown. Place the Integral into the reagent area and let it stand there for 30 min. During this time, the magnetic microbeads are automatically agitated and completely resuspended.

**Do not interchange Nano Magnetic Microbeads from different reagents!**

4.3 Storage of the Reagents Integral

- **Sealed:** Stored at 2-8°C until the expiry date.
- **Opened:** Stable for 4 weeks. After this period, it is still possible to keep on using the Reagent Integral provided that the controls are found within the expected ranges.
- Keep upright.
- Keep away from direct sunlight.

5. Origin of Calibrators

Calibrators in the reagent kit are from Sigma. Biological root: synthetic materials, processed by HPLC purification, with a purity ≥94%. No HBsAg, anti-HCV, and anti-HIV are found.

6. Calibration

6.1 2 point recalibration

Via the measurement of calibrators, the predefined master curve is adjusted (recalibrated) to a new, instrument-specific measurement level with each calibration.

6.2 Frequency of Recalibration

- After each exchange of lot (Reagent Integral or Starter Reagents).
- Every week and/or each time a new Integral is used (recommendation).
- After each servicing of the Maglumi Fully Auto analyzer.
- If controls are beyond the expected range.

7. Sample Collection, Material and Storage

- Collect samples using standard procedures.
- Sample material: serum.
- Store at 2-8°C: 24 hours.
- For longer storage periods: freeze to below -20°C.
- Avoid repeated freezing and thawing cycles.
- Stored samples should be thoroughly mixed prior to use (Vortex mixer).

Vacuum tubes

- (Blank) tubes are recommended type for collecting samples.
- If plasma sample is needed, EDTA tube is conformed has no effect on the results RLU.
- (c) Liquemin Sodium tube is found to increase the sample RLU and cause test results deviation.
- Please ask SNBSE for advice if special additive must be used in the sample blood.

8. WARNING AND PRECAUTIONS FOR USERS

- For use in IN-VITRO diagnostic procedures only.
- Do not interchange reagents from different lots. Do not use kit components beyond their labeled expiry date.
- All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each country. Disposable materials must be incinerated; liquid waste must be decontaminated with sodium hypochlorite at a final concentration of 5% for at least half an hour. Any materials to be reused must be autoclaved using an overkill approach (USP24,2000.p.2143). A minimum of one hour at 121°C. is usually considered adequate, though the users must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.
- The calibrators in this kit are prepared from bovine serum products. However, because no test method can offer complete assurance that HIV, Hepatitis B Virus or other infectious agents are absent, these reagents should be considered a potential biohazard and handled with the same precautions as applied to any serum or plasma specimen.

9. Test Procedure

To ensure proper test performance, strictly adhere to the operating instructions of the Maglumi Fully Auto analyzer. Each test parameter is identified via a RFID Tag on the Reagent Integral. For further information please refer to the Maglumi Fully Auto Operator’s Manual.

<table>
<thead>
<tr>
<th>100μl</th>
<th>Sample, calibrator or controls</th>
</tr>
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10. Quality Control

- Observe quality control guidelines for medical laboratories.
- Use suitable controls for in-house quality control.

11 Results

11.1 Calculation of Results

- The analyzer automatically calculates the 25-OH VITAMIN D concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in nm/l. For further information please refer to the Maglumi Fully Auto Operator’s Manual.

11.2 Interpretation of Results

- Results may differ between laboratories due to variations in population and test method. Each laboratory should establish its own reference range.

12. Limitations of the procedure

12.1 Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions. A skilful technique and strict adherence to the instructions is necessary to obtain reliable results. Procedural directions must be followed exactly and careful technique must be used to obtain valid results. Any modification of the procedure is likely to alter the results. Bacterial contamination or repeated freeze-thaw cycles may affect the test results.

13. Performance Characteristics

13.1 Accuracy

Consider High Calibrator of known concentration as a sample, dilute it by 1:2 ratio with diluent, and measure its diluted concentration for 10 times. Then calculate the recovery of measured concentration and expected concentration. The recovery should be within 90% -110%.

13.2 Precision

Intra-assay coefficient of variation was evaluated on Calibrator High repeatedly measured 10 times in the same assay, calculating their coefficient of variation, the results should <10%. Inter-assay coefficient of variation was evaluated on three batches of kit, repeatedly measured 10 times of Calibrator High, calculating three batches of kit for Calibrator High between the measured values of the coefficients of variation, the results should <15%.

13.3 Sensitivity

The sensitivity is defined as the concentration of 25-OH VITAMIN D equivalent to the mean RLU of 20 replicates of the zero standard plus two standard deviations corresponding to the concentration from the standard curve. The sensitivity is typically less than 3ng/ml.

13.4 Specificity

The specificity of the 25-OH VITAMIN D assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes. When 3-epi-25 OH Vitamin D3=100 ng/ml, the detection results of 25-OH VITAMIN D ≤3ng/ml.

13.5 Linearity

Conduct a logarithmic transform to the RLU value and concentration value of 6 standards. After a double logarithmic fitting, the absolute value of its linearity should exceed 0.9800.

14 References