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WS

**INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

WS/T 677-2020

**Screening methods for vitamin D deficiency in the population
(English Translation)**

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Foreword

This standard was drafted in accordance with the rules given in GB/T 1.1-2009.

Drafting organizations of this standard. Institute of Nutrition and Health, Chinese Center for Disease Control and Prevention, Ningbo Center for Disease Control and Prevention, Shenzhen Chronic Disease Prevention Center, Shenzhen Institute of Metrology and Quality Inspection, Nanjing Medical University, COFCO Nutrition and Health Research Institute.

1 Scope

This standard specifies the indicators, reference judgment values and detection methods for the screening of population vitamin D deficiency and deficiency.

This standard applies to the determination of the nutritional status of vitamin D in the population.

2 Normative references

The following documents are indispensable for the application of this document. For dated reference documents, only the dated version applies to this document.

For undated references, the latest version (including all amendments) applies to this document.

Preparation of preparations and products used in GB/T 603 chemical reagent test method
GB/T 6682 Analytical laboratory water specifications and test methods WS/T 225 Collection and processing of blood samples for clinical chemistry tests

3 Terms and definitions

The following terms and definitions apply to this document.

25-hydroxyvitamin D 25-hydroxyvitamin D; 25(OH)D the main circulating form of vitamin D in the blood has good stability and is a recognized reliable indicator for evaluating the nutritional status of human vitamin D.

There are two forms. 25(OH)D₂, 25(OH)D₃, 25(OH)D₃ is the main form of vitamin D in the blood.

Vitamin D deficiency when the human serum (or plasma) 25-hydroxyvitamin D content is lower than the reference judgment value of deficiency, it can be judged as vitamin D deficiency.

Vitamin D insufficiency when the human serum (or plasma) 25-hydroxyvitamin D content is lower than the reference judgment value of the normal population, but higher than the lack of reference judgment value at this time, it can be judged as insufficient vitamin D.

4 Judgment index and reference judgment value of population vitamin D nutritional status

See Table 1 for the judgment index and reference judgment value of vitamin D nutritional status of the population.

5.1 Collection and storage of blood samples

Follow the method specified in Appendix A.

5.2 The first method. liquid chromatography tandem mass spectrometry Follow the method specified in Appendix B.

5.3 Method 2. Chemiluminescence immunoassay

Follow the method specified in Appendix C.

5.4 The third method. enzyme-linked immunoassay Follow the method specified in Appendix D.

Appendix A

(Normative appendix) Collection and preservation of blood samples A.1 Specimen collection A.1.1 Collection of blood samples. fasting venous blood, the blood collection method is based on WS/T 225 and the requirements of the detection method to collect 1 mL of fasting venous blood.

A.1.2 Blood collection tube. According to the required serum or plasma, use different blood

collection tubes for packaging.

A.2 Serum and plasma preservation the specimen can be stable for 7 days at 2 °C to 8 °C, and stable for 12 months at -20 °C. If the specimen cannot be tested in time, it is recommended Store specimens below -70 °C, freeze-thaw only once.

Appendix B

(Normative appendix) Liquid chromatography tandem mass spectrometry B.1 Principle the 25(OH)D₂ and 25(OH)D₃ in serum (plasma) were precipitated with methanol/acetonitrile, extracted with n-hexane, dried with nitrogen, and dried with initial the mobile phase is reconstituted, separated by liquid chromatography, detected by tandem mass spectrometry in multiple reaction monitoring mode (MRM), and quantified by isotope internal standard the method can measure 25(OH)D₂ and 25(OH)D₃ respectively.

B.2 Reagents and solutions Unless otherwise specified, all reagents in this method are analytically pure, the water complies with the first grade water regulations in GB/T 6682, and the solution complies with GB/T 603 Preparation.

B.2.1 Reagents

B.2.1.1 25(OH)D₂ standard product (C₂₈H₄₄O₂, CAS. 21343-40-8). purity > 98% or certified standard material.

B.2.1.2 25(OH)D₃ standard substance (C₂₇H₄₄O₂, CAS. 19356-17-3). Purity > 98% or certified standard substance.

B.2.1.3 Isotope internal standard [2H₃]-25(OH)D₂ standard product (C₂₈H₄₁ H₃O₂, CAS. 1217467-39-4). Purity > 98%.

B.2.1.4 Isotope internal standard [2H₃]-25(OH)D₃ standard (C₂₇H₄₁ H₃O₂, CAS. 140710-94-7). Purity > 98%.

B.2.1.5 Methanol (CH₃OH). chromatographically pure.

B.2.1.6 n-hexane (C₆H₁₄). chromatographically pure.

B.2.1.7 Formic acid (HCOOH). chromatographically pure.

B.2.1.8 Bovine serum albumin (BSA). purity ≥98%.

B.2.1.9 Nitrogen (N₂). Purity ≥99.9%.

B.2.1.10 Formic acid-water solution. 0.1%.

B.2.1.11 Formic acid-methanol solution. 0.1%.

B.2.1.12 Acetonitrile (CH₃CN). chromatographically pure.

B.2.1.13 BSA bovine serum albumin aqueous solution. 1%.

B.2.1.14 Precipitant. methanol acetonitrile (11).

B.2.1.15 Standard stock solution (10 µg/mL). Weigh 1 mg of 25(OH)D₂ and 25(OH)D₃ standards (accurate to 0.01 mg) in 2 100 mL volumetric flasks, dissolve with methanol and dilute to the mark. The concentration of 25(OH)D₂ and 25(OH)D₃ in this solution is 10 µg/mL. Melt After transferring the solution to the reagent bottle, store it in the dark at -20°C for later use.

B.2.1.16 Isotope internal standard stock solution (10 µg/mL). Weigh the isotope internal standards [2H₃]-25(OH)D₂ and [H₃]-25(OH)D₃ respectively 1 mg (accurate to 0.01 mg) in 2 100 mL volumetric flasks, dissolved in methanol and dilute to the mark. In this solution [2H₃]-25(OH)D₂ and the concentration of H₃]-25(OH)D₃ is 10 µg/mL. After the solution is transferred to the reagent bottle, store it in the dark at -20°C for later use.

B.2.1.17 Standard series solutions. accurately pipette 25(OH)D₂ (10 µg/mL) and 25(OH)D₃ (10 µg/mL) 5 µL, 10 µL, 25 µL, 50 µL, 125 µL, 250 µL and 500 µL were placed in 7 10 mL volumetric flasks, diluted with methanol and made up to the mark. This series of solutions the concentrations of 25(OH)D₂ and 25(OH)D₃ are 5 ng/mL, 10 ng/mL, 25 ng/mL, 50 ng/mL, 125 ng/mL, 250 ng/mL and 500 ng/mL.

B.2.1.18 Mixed isotope internal standard solution (100 ng/mL). accurately draw the isotope internal standard [2H₃]-25(OH)D₂ stock solution (10 µg/mL) and [2H₃]-25(OH)D₃ stock solution (10 µg/mL) 100 µL each in a 10 mL volumetric flask, dilute with methanol and make up to the mark.

The isotope internal standard of this solution [2H₃]-25(OH)D₂ and [the concentration of H₃]-25(OH)D₃ is 100 ng/mL. After the solution is transferred to the reagent bottle, Store in the dark at -20°C for later use.

B.2.2 Apparatus and equipment B.2.2.1 Analytical balance, sensitivity. 0.0001 g.

B.2.2.2 Analytical balance, sensitivity. 0.00001 g.

B.2.2.3 Liquid chromatography-tandem mass spectrometer.

B.2.2.4 Nitrogen blowing instrument or vacuum centrifugal dryer.

B.2.2.5 Centrifuge. centrifugal force >=12000xg.

B.2.2.6 Vortex mixer.

B.2.2.7 Pipette gun or pipette.

B.3 Analysis steps

B.3.1 Sample preparation Take.200 µL of serum (plasma) sample into a 2 mL centrifuge tube, add 20 µL of mixed isotope internal standard solution, and vortex for 30 s. plus Add 400 µL of precipitant (methanol acetonitrile, 1 1), vortex and shake at room temperature for 30 s; add 1.2 mL of n-hexane, and vortex at room temperature 5 min, then centrifuge for 5