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

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**Method for Vitamin A deficiency screening
(English Translation)**

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Table of Contents

Foreword

1 Scope

2 Normative references

3 Terms and definitions

4 Criteria for borderline deficiency and deficiency of vitamin A in the population

5 Criteria for determining the level of public vitamin A deficiency in the population

6 Determination of serum (plasma) vitamin A

Foreword

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

The main drafting organizations of this standard. Institute of Nutrition and Health, Chinese Center for Disease Control and Prevention, Capital Institute of Pediatrics, Peking University Health Science Center Public Health College, Zhejiang Provincial Center for Disease Control and Prevention.

1 Scope

This standard specifies the indicators and detection methods for the screening of the population for vitamin A deficiency and the corresponding thresholds for vitamin A deficiency.

This standard applies to the determination of vitamin A deficiency in the population. The clinical diagnosis of individual vitamin A deficiency needs to be combined with clinical practice.

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

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.

Vitamin A vitamin A

Retinol

Generic term for Pionone derivatives. A fat-soluble vitamin. There are two kinds of vitamin A1 and vitamin A2 3.2 Vitamin A deficiency the level of vitamin A in the human body is not enough to maintain normal physiological functions, and the level of retinol in the serum (plasma) of children (6 years old and below) is lower than $0.35\mu\text{mol/L}$, less than $0.70\mu\text{mol/L}$ for children over 6 years old and adults, and pathological changes of eyes and skin may occur.

Marginal vitamin A deficiency the level of vitamin A in the human body can maintain normal physiological functions, but the level of retinol in the serum (plasma) increases after vitamin A supplementation.

4 Criteria for borderline deficiency and deficiency of vitamin A in the population

The content of retinol per unit volume of serum (plasma) is used as a screening indicator for vitamin A deficiency. When the measured serum (plasma) retinol contains when the amount is less than the corresponding reference value, it is judged as the corresponding borderline deficiency or deficiency of vitamin A. See Table 1 for the judgment index and judgment threshold.

Note: Conversion factor of 1mol retinol = 286.45 g retinol.

5 Criteria for determining the level of public vitamin A deficiency in the population

In the assessed population of 1 year and older, calculate the proportion of people with serum (plasma) retinol content $< 0.70\mu\text{mol/L}$, according to vitamin A the lack of public health problem criteria determines the severity of the public health problem of vitamin A deficiency in this population. Determine the problem level and the judgment value is shown in Table 2.

6 Determination of serum (plasma) vitamin A

Measure according to the serum (plasma) retinol determination method specified in Appendix A-high performance liquid chromatography (HPLC).

Appendix A

(Normative appendix) Serum (plasma) retinol determination method-high performance liquid chromatography (HPLC) A.1 Principle Serum (plasma) was added with internal standard retinol acetate, extracted with n-hexane, and separated by high performance liquid

chromatography C18 reverse column After separation, it is quantitatively detected by UV detector.

A.2 Reagents and materials Unless otherwise specified, all chromatographic reagents and distilled water or equivalent purity water (H₂O) are used in this method.

A.2.1 Anhydrous ethanol (C₂H₆O).

A.2.2 Methanol (CH₄O).

A.2.3 n-hexane (C₆H₁₄).

A.2.4 Nitric acid (HNO₃).

A.2.5 Standard Retinol (C₂₀H₃₀O).

A.2.6 Standard Retinol Acetate (C₂₂H₃₂O₂).

A.2.7 High-purity nitrogen (N₂).

A.3 Instruments

A.3.1 High performance liquid chromatograph with UV spectrophotometer.

A.3.2 Nitrogen blowing instrument.

A.3.3 Centrifuge. speed $\geq$ 5000 r/min.

A.3.4 Balance. Sensitivity is 0.1 mg.

A.3.5 Ultraviolet spectrophotometer.

A.4 Sample collection and storage refer to WS/T 225 for venous blood collection, serum (plasma) separation and blood sample storage.

Note: The collection and pre-processing of blood samples must be carried out in a dark or red light environment to avoid changes in the content of retinol in the blood samples.

A.5 Analysis steps

A.5.1 Preparation of retinol standard stock solution A.5.1.1 Take a certain amount of standard retinol, put it into a 10 mL volumetric flask, dissolve it in a small amount of absolute ethanol, and dilute it to 10 mL.

Into retinol standard stock solution.

A.5.1.2 Draw 100 μ L of the retinol standard stock solution, place it in a 10 mL volumetric flask, and add absolute ethanol to make the volume to 10 mL (repeat twice).

A.5.1.3 Use an ultraviolet spectrophotometer to detect the content of the retinol standard stock solution at a wavelength of 325 nm. The blank reagent is anhydrous ethyl acetate. alcohol. The specific absorption coefficient was used to calculate the concentration of

retinol in the standard stock solution.

A.5.1.4 The retinol content in the stock solution is calculated according to formula (A.1).

A.5.2 Preparation of retinol acetate internal standard A.5.2.1 Using a 0.1 mg balance, accurately weigh 82.0 mg of the standard retinol acetate, and pour it into a 10 mL volumetric flask.

After the alcohol is fully dissolved, the volume is adjusted to 10 mL, and the concentrated solution of retinol acetate internal standard is prepared.

A.5.2.2 Use a high performance liquid chromatograph to detect the peak area of the concentrated solution of retinol acetate internal standard at 325 nm, and dilute it with absolute ethanol.

Release to a working solution of retinol acetate internal standard at a concentration of approximately 6 µg/mL.

A.5.3 Drawing of standard curve A.5.3.1 Preparation of retinol standard curve Respectively draw the calibrated retinol standard stock solution into a 10 mL brown volumetric flask, and then add an equal volume of retinol acetate internal standard to work Place the solution in a brown volumetric flask and dilute to the mark with absolute ethanol so that the concentration of the retinol standard solution is 1.00 µg/mL, 0.50 µg/mL, 0.30 µg/mL, 0.20 µg/mL, 0.10 µg/mL and 0.05 µg/mL. To be tested, get on the machine.

Note: All standard products are stored in a -20°C low-temperature refrigerator.

A.5.3.2 Reference conditions for high performance liquid chromatography the reference conditions of HPLC should meet the following requirements.

--Pre-column. 4.0 mmx4.5 cm, 10 µm.

--Analytical column. C18 column, 4.6 mmx25.0 cm, 5 µm.

--Mobile phase. methanol.water=98.2.Mix the mobile phase thoroughly before use, and degas before use.

--Flow rate. 1 mL/min.

--Detection wavelength. 325 nm.

--Column temperature. 28°C±1°C.

--Sampling volume. 20 µL.

A.5.3.3 Drawing the standard curve of retinol Respectively take the retinol standard series solution from low to high for determination, and add retinol with the same concentration as the internal standard in the standard for each determination the alcohol acetate internal standard was used for the determination. Take the concentration of retinol standard series solution as the abscissa, and take the peak area of retinol and the internal standard of