Supplement 1.　Recommendations for the clinical determination of blood lipids.

Blood lipid measurement is an important component of the management of dyslipidemia. The accuracy of the detection results is the basic requirement for the effective management of dyslipidemia. Many factors influence the accuracy of blood lipid measurement results, including test participants and specimen conditions, measurement methods, instruments and reagents, and measurement manipulation. According to the current and relevant recommendations of the Chinese Society of Laboratory Medicine of the Chinese Medical Association, the relevant industrial and national standards in China, actual blood lipid measurement conditions in China, and the combined opinions of the Joint Committee of the Revision of the Chinese Guidelines for the Management of Dyslipidemia in Adults, the recommendations concerning the measurement of blood lipid items [including serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoproteins (Apo) A1, Apo B, and lipoprotein (a)] should promote the further standardization of blood lipid measurement in China and ensure the effective management of dyslipidemia.

1　Participant preparation and specimen collection

Many pre-analysis factors influence blood lipid levels including biological factors such as between-individual factors, including gender, age, and race; behavioral factors, such as diet, obesity, smoking, stress, alcohol drinking, coffee drinking, and exercise; clinical factors, such as secondary disease (i.e., endocrine or metabolic diseases, kidney diseases, hepatobiliary disease, and others) and drug induction (i.e., antihypertensive drugs, immunosuppressive agents, and estrogen); and specimen collection and processing factors, such as fasting status, blood concentration, anticoagulants, preservatives, capillary and venous blood, and specimen storage. The following measures to reduce the influence of controllable pre-analysis factors on blood lipid measurement results are recommended:

(1) Participants should be in a stable metabolic state before specimen collection and maintain general eating habits and a stable body weight for at least 2 weeks;

(2) Participants should not perform strenuous physical activity within 24 h of specimen collection;

(3) Participants should fast for approximately 12 h before specimen collection;

(4) Venous blood should be used as a specimen for blood lipid measurement. Participants should sit and rest for at least 5 min before blood drawing. Except under special conditions, participants should sit for blood drawing;

(5) A tourniquet should not be more than 1 min during venous puncture;

(6) Blood specimens should remain sealed, and shaking should be avoided;

(7) Serum samples should be used for blood lipid analyses. Blood specimens are centrifuged within 1−2 h to separate the serum (tubes containing anti-coagulants can be centrifuged within a briefer time period); and

(8) Serum samples should be analyzed in time to avoid storage. If the sample must be stored, then they should remain sealed. They can be stored at 4°C for up to 3 day; samples should be stored below −70°C for long-term storage.

2　Selection of detection methods

The principles of all blood lipid measurement methods differ. The analytic performance, operability, and analyses cost are also different. Routine blood lipid measurement should select appropriate measurements based on relevant conditions.

2.1　Serum TC detection

The measurements of serum TC include chemical, chromatography, and enzymatic methods. Enzymatic methods are the simplest; they are easy to automate and show excellent analytic performance. Therefore, they are currently the most commonly used methods for routine TC measurements. Other methods are currently only used under certain conditions (e.g., specific chemical methods and chromatography are used as reference methods). Enzymatic methods are recommended for routine TC measurements.

2.2　Serum TG detection

Methods for serum TG measurement include chemical, chromatography, and enzymatic methods. Currently, enzymatic methods are the most commonly used routine TG measurements. Currently, most TG enzymatic methods measure total glycerol. Some enzymatic methods deduct free glycerol. Enzymatic methods are recommended for routine serum TG measurement. Generally, the total glycerol measurement

can be used. When necessary, measurement methods that eliminate the influence of free glycerol should be considered.

2.3 Serum HDL-C detection

Many methods for HDL-C measurement exist, and these methods can roughly be divided into ultracentrifugation, electrophoresis, chromatography, precipitation, and homogeneous methods. Previous routine HDL-C measurements primarily used precipitation methods. The precipitation methods that pass strict certification can achieve a high analytic specificity. However, their major shortcomings are that specimens should undergo processing methods such as precipitation and centrifugation in advance. High TG levels easily affect the results. The current major methods for routine HDL-C measurement are homogenous methods including clearance, polyethylene glycol (PEG)-modified enzyme, selective inhibition, and immune separation methods. The greatest advantages of homogeneous methods are ease of use, no sample processing is required, and they show excellent analytic performance. However, some methods might have a specificity issue. Homogeneous methods are recommended for routine serum HDL-C measurement.

2.4 Serum LDL-C detection

The measurement methods for serum LDL-C include ultracentrifugation, electrophoresis, chromatography, formula calculation, precipitation, and homogeneous methods. The major methods are formula calculation, precipitation, and homogeneous methods. Formula calculation methods were once the most commonly used LDL-C measurement worldwide. They remain extensively used in certain countries. The most used formula is the Friedewald formula, where LDL-C = TC − HDL-C − TG/5 (mg/dL) or LDL-C = TC − HDL-C − TG/2.2 (mmol/L). The greatest advantages of this method are that no detection is required and the formula is easy to calculate. When TG is < 2.8 mmol/L (250 mg/dL), a certain level of reliability is present. The limitations are that it cannot be used when TG > 4.5 mmol/L (TG > 400 mg/dL); alternatively, the measurement quality of three indicators (TC, TG and HDL-C) affect the LDL-C results in some specimens of abnormal lipoproteinemia. Some countries formerly used precipitation methods for LDL-C measurement. However, due to their limited specificity and complicated manipulation, their application is not extensive. Homogeneous methods are currently the major methods for LDL-C measurement in China; these methods include clearance, calixarene, solubilization reaction, and protecting reagent methods. This group of methods is easy to use and can be applied to analyze high TG samples. However, some methods might have specificity issues. Homogeneous methods are recommended for routine LDL-C measurement.

2.5 Serum Apo A1, Apo B, and Lp (a) detection

The measurement of serum Apo A1, Apo B, and Lp (a) is based on immunochemistry principles. Early measurements primarily used immunoelectrophoresis and radioimmuno- and enzyme-linked immunosorbent assays. These methods are associated with complicated manipulations, and their analysis performance is limited; therefore, they are currently rarely used. The current immunoturbidimetric assay primarily used includes transmission turbidimetry and nephelometry. These methods are easy to use and show excellent performance. Some Lp (a) measurements might have obvious specificity issues. Immunoturbidimetric assay is recommended for routine measurements of serum Apo A1, Apo B, and Lp (a).

3 Selection of analysis systems

The specific instrument, reagents, standard materials, and working parameters required for the operation of the above methods are known as "analysis systems". Currently, routine blood lipid measurements generally use commercial instruments, reagents, and calibration materials. Many brands exist; therefore, many analysis systems are available to perform the same method. Different analysis systems usually show different analysis performances. Therefore, the selection of a reliable analysis system is the key to ensure the quality of blood lipid analyses.

3.1 Types of analysis systems

The automation level of analytic instruments can be divided into fully automatic (automatic biochemical analyzers), semi-automatic (semi-automatic analyzers), and manual (spectrophotometer) analysis systems. In addition to analytic instruments, reagents, and calibration materials, semi-automatic and manual analysis systems also include equipment and apparatuses for liquid transfer and incubation. Currently, most clinical laboratories in China use fully automatic analysis systems. Certain small laboratories might use semi-automatic analysis systems. Automation degree negatively predicts the number of influencing factors. Fully automatic analysis systems are recommended for routine blood lipid measurements. When appropriate, semi-automatic analysis systems can be used.

Based on the sources of instruments, reagents, and calibration materials, analysis systems can be divided into 3 types: (1) closed system (i.e., the instruments, reagents, and calibration materials are from the same vender; they are

used in matches; and the working parameters are built-in); (2) open system (i.e., the reagents and calibration materials are from the same vender and used in matches; the instruments are selected elsewhere; and the reagent venders usually provide the parameters); and (3) combination system (i.e., the instruments, reagents, and calibration materials are from different vendors or institutions and assembled by laboratories; and the laboratories established the working parameters). Currently, all three types of analysis systems are extensively used in China. Laboratories can choose their analysis system based on their specific conditions.

3.2 Quality and technique indicators of analysis systems

The selected analysis systems should conform to the following quality and technique indicators.

3.2.1 Precision, correctness, and accuracy

Precision refers to the degree of consistency among results obtained from the repeated analysis of the same sample across multiple independent analyses. This variable reflects the random error of the analysis system, and it is expressed as the variation coefficient. The variation coefficients of serum measurements of TC, TG, HDL-C, LDL-C, Apo A1, Apo B, and Lp (a) should be lower than 3%, 5%, 4%, 4%, 3%, 3%, and 4%, respectively.

Correctness refers to the difference between the mean of the results obtained from repeated analyses of the same sample across multiple independent detection analyses and the target value. This variable reflects the system error of the analysis system, and it is expressed as a deviation (B). The target value usually refers to the definite value of the reference (standard) material or the value measured using reference methods. The measured deviations of serum TC, TG, HDL-C, LDL-C, Apo A1, Apo B, and Lp (a) should be within the ranges of $\pm 3\%$, $\pm 5\%$, $\pm 5\%$, $\pm 4\%$, $\pm 5\%$, $\pm 5\%$, and $\pm 10\%$, respectively.

Accuracy refers to the maximum difference between the obtained results from the analysis of many representative samples within a single time period across multiple independent detection analyses and the target value, which is expressed using total error. The target value generally refers to the measurement value using reference methods or other reliable methods. Assuming that the analysis system shows excellent specificity, accuracy is determined via precision and correctness (expressed using the formula total error = absolute value of deviation + 1.96 × variation coefficient). The total errors of serum TC, TG, HDL-C, and LDL-C should be lower than 9%, 15%, 13%, and 12%, respectively.

Precision, correctness, and (especially) accuracy are the main quality indicators of the analysis system. Currently, the precision of most blood lipid analysis systems in China are excellent. Some analysis systems might have correctness and accuracy issues.

3.2.2 Specificity

Specificity is an important factor affecting accuracy. The analysis system should have the ability that it only functions with regard to target blood lipid indicators and is not influenced by other serum components. Currently, the TC and TG analysis systems in China show excellent specificity. Some lipoprotein and Apo analysis systems might have specificity issues.

3.2.3 Calibration

Calibration is the decisive factor of accuracy. The analysis of the value of the calibration materials in the system should be able to track clinical specimen measurement results to the existing reference system.

3.2.4 Detection range

The detection range of the analysis system should cover the following blood lipid ranges: TC, 2–10 mmol/L; TG, 0.3–10 mmol/L; HDL-C, 0.3–2.5 mmol/L; LDL-C, 0.5–7 mmol/L; ApoA1 0.5–2.0 g/L; ApoB 0.5–2.0 g/L and Lp (a), 5–800 mg/L.

3.3 Validation of analysis system performance

Any newly selected analysis system should receive a performance validation before being used with regard to a clinical sample to ensure that its performance can meet the aforementioned quality technical indicators. The specific validation methods should reference relevant industrial standards or literature.

3.4 Blood lipid analysis

A blood lipid analysis for clinical specimens is performed using a validated analysis system. Analysis manipulation is performed according to the procedures regulated in the instructions of the analysis system or reagents.

4 Quality control and assurance

Clinical laboratories should regulate the working conditions and procedures of all major links in blood lipid measurement. Blood lipid measurement should be performed according to regulations.

In Chinese medical institutions, specimen collection and detection analysis research usually occurs in different departments. Different departments should communicate closely to ensure the effective implementation of relevant working

procedures to try to minimize the effect of pre-analysis factors on blood lipid measurement.

Clinical laboratories should select blood lipid measurement methods and analysis systems (primarily the brands of reagents and calibration materials) with reliable performance according to experience, industrial communications, and the scientific literature. They should try to keep the same type of analysis system, which should not be changed arbitrarily or frequently.

Clinical laboratories should perform internal quality control. Quality control samples should be appropriate for blood lipid analysis, homogeneous, and stable. The concentrations should approximate the main medical decision levels and have at least two levels. The same type of quality control samples should be used for as long as possible and should not be changed frequently. The analysis of quality control samples should be performed at least once for each batch of detection analyses.

Clinical laboratories should regularly participate in nationally or regionally recognized quality assessment plans across laboratories.

5 Result report and explanation

Clinical laboratories should report TC, TG, HDL-C, and LDL-C measurement results using the Chinese legal unit of measurement (mmol/L). When necessary, results using the traditional unit (mg/dL) can be reported.

Blood lipid examination reports should indicate the major medical decision levels regulated by the Chinese Guidelines for the Management of Dyslipidemia.

The explanation of blood lipid detection results should consider analytic variations and the influence of biological variations among individuals. If blood lipid results approximate the medical decision level, then a decision should be made based on multiple blood lipid measurement results.