China Health and Nutrition Survey (CHNS)

Manual for
Specimen Collection and Processing

China-Japan Friendship Hospital, the Ministry of Health
July, 2009
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1 PREFACE

The China Health and Nutrition Survey (CHNS) is a multi-site, interdisciplinary epidemiologic study in China led by Dr. Barry M. Popkin from University of North Carolina since 1989, and is sponsored by the National Institutes of Health (NIH). This multi-regional follow-up research project collects data regarding different social economic aspects: family occupation, income and welfare, time arrangements, dietary and nutritional status, daily activities, health status, health service usage, marriage and pregnancy of women at childbearing age, family size and composition, life planning, care for children and seniors, housing conditions, land ownership, household expenditures, etc. Personal health data are recorded in great detail, including detailed measurement of dietary intake and physical activities, smoking and alcohol consumption data, demographic data, blood pressure and specified clinical data. Its goals include observing dietary structure and health status of residents, as well as identifying socio-economic and environmental effects on their nutritional intake, physical activity and health.

The study involves nine provinces (Hei Long Jiang, Liao Ning, Jiang Su, Shan Dong, He Nan, Hu Bei, Hu Nan, Guang Xi, and Gui Zhou), 54 counties or cities, 216 communities, approximately 4500 families, and 20,000 individuals. Information collected through the project include (1) community information, family information, personal information, dietary and physical examination results, and geographical location data; (2) blood collection, preservation and analysis, as well as toenail sample collection.

This international tripartite cooperation project has been approved by the Ministry of Health and is now run by the University of North Carolina (US), China Disease Prevention and Control Center [responsible for (1)], and China-Japan Friendship Hospital [responsible for (2)] (China). Later two has joined together as sponsor from China for future investigation and analysis.

The manual covers the entire process of biological samples collection, including sample collection, processing, preservation, and related analytical tests, which effectively ensures quality control of the sample collection process.

This manual is written by CHNS project team from China-Japan Friendship Hospital, Ministry of Health, and is only expected to be used within the program.
2 INVESTIGATION METHOD

2.1 Scope of the Survey and Participants

This survey performs a follow-up investigation regarding all participants involved in seven years’
survey (1989-2006), including possible increase or decrease in number. Joint team of Biological
sample collection from China-Japan Friendship Hospital of the Ministry of Health is responsible for
providing complete and clear information of all surveyed individuals, and timely communication
with survey team members Chinese Centre for Disease Control and Prevention is expected.

2.2 Staff Training

Training of the survey is composed of concentrative national-level training and subsidiary
provincial-level training.

2.2.1. Trainers

China-Japan Friendship Hospital is responsible for the national-level training, and provincial co-
operators are responsible for the provincial–level training.

2.2.2. Trainees

Trainees of the national-level training are provincial project managers in charge of on-site specimen
collection. Participants of the national-level training are also responsible for conducting provincial-
level training.

2.2.3. Course pack

- *Manual for Specimen Collection and Processing* by China-Japan Friendship Hospital
- Other related software and handouts

2.2.4. Objects and methods

National-level training involves lectures, field demonstrations, field operations and other forms of
teaching methods. The combination of theoretical methodology and practices improves trainees’
ability of manipulation and awareness of standardization. Provincial-level training is constructed in a
similar way.

<table>
<thead>
<tr>
<th>Schedule</th>
<th>Requirements</th>
<th>Methods</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Introduction to investigation method</td>
<td>master</td>
<td>Lecture; Multimedia teaching</td>
<td>0.5 hours</td>
</tr>
<tr>
<td>2. Biological sample collection work flow</td>
<td>master</td>
<td>Lecture;</td>
<td>1.5 hours</td>
</tr>
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</table>
### Multimedia teaching

<table>
<thead>
<tr>
<th>Topic</th>
<th>Teaching Method</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. Preparation of specimen collection</td>
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</tr>
<tr>
<td></td>
<td>Lecture; Multimedia teaching; Group exercises; On-site examination</td>
<td></td>
</tr>
<tr>
<td>4. On-site control procedures</td>
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<td>2 hours</td>
</tr>
<tr>
<td></td>
<td>Group teaching; Group exercises; On-site field simulation; On-site examination</td>
<td></td>
</tr>
<tr>
<td>5. Questionnaires and forms</td>
<td>master</td>
<td>1 hour</td>
</tr>
<tr>
<td></td>
<td>Lecture; Group exercises; On-site field simulation; On-site examination</td>
<td></td>
</tr>
<tr>
<td>6. Quality control</td>
<td>master</td>
<td>1 hour</td>
</tr>
<tr>
<td></td>
<td>multimedia teaching</td>
<td></td>
</tr>
</tbody>
</table>

### 2.3 Coding Rules and Directions

#### 2.3.1 Community Coding Rules

Since the survey involves nine provinces, more than two hundred sites in cities and counties, the following rule of coding is provided for sample records. Each site is required to accurately label correct code on questionnaires, documents, forms, and sample storage boxes upon completion of specimen collection. For example: If the sample is collected from Guan Ku Lane, Bei Ta Street, Ping Jiang District, City of Suzhou, Jiangsu Province, the proper community code to be labeled on is "321101".

1. **Province Code**

<table>
<thead>
<tr>
<th>Province</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liao Ning</td>
<td>21</td>
</tr>
<tr>
<td>Hei Long Jiang</td>
<td>23</td>
</tr>
<tr>
<td>Jiang Su</td>
<td>32</td>
</tr>
<tr>
<td>Shan Dong</td>
<td>37</td>
</tr>
<tr>
<td>He Nan</td>
<td>41</td>
</tr>
<tr>
<td>Hu Bei</td>
<td>42</td>
</tr>
<tr>
<td>Hu Nan</td>
<td>43</td>
</tr>
<tr>
<td>Guang Xi</td>
<td>45</td>
</tr>
<tr>
<td>Gui Zhou</td>
<td>52</td>
</tr>
</tbody>
</table>

2. **Urban/Rural Code**

- Urban: 1
- Rural: 2
(3) City/County level code

<table>
<thead>
<tr>
<th>Level</th>
<th>Tier</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>city</td>
<td>First tier</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Second tier</td>
<td>2</td>
</tr>
<tr>
<td>county</td>
<td>First tier</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Second tier</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Third tier</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Fourth tier</td>
<td>4</td>
</tr>
</tbody>
</table>

(4) Urban residential committee/village and town level code

<table>
<thead>
<tr>
<th>Level</th>
<th>Tier</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban residential committee</td>
<td>No.1 committee</td>
<td>01</td>
</tr>
<tr>
<td></td>
<td>No.2 committee</td>
<td>02</td>
</tr>
<tr>
<td></td>
<td>No.3 rural committee</td>
<td>03</td>
</tr>
<tr>
<td></td>
<td>No.4 rural committee</td>
<td>04</td>
</tr>
<tr>
<td></td>
<td>No.5 committee</td>
<td>05</td>
</tr>
<tr>
<td></td>
<td>No.6 committee</td>
<td>06</td>
</tr>
<tr>
<td></td>
<td>No.7 rural committee</td>
<td>07</td>
</tr>
<tr>
<td></td>
<td>No.8 rural committee</td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>No.9 committee</td>
<td>09</td>
</tr>
<tr>
<td></td>
<td>No.10 committee</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>No.11 rural committee</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>No.12 rural committee</td>
<td>12</td>
</tr>
<tr>
<td>Village and town committee</td>
<td>No.1 village</td>
<td>01</td>
</tr>
<tr>
<td></td>
<td>No.2 village</td>
<td>02</td>
</tr>
<tr>
<td></td>
<td>No.3 village</td>
<td>03</td>
</tr>
<tr>
<td></td>
<td>No.4 village</td>
<td>04</td>
</tr>
<tr>
<td></td>
<td>No.5 village</td>
<td>05</td>
</tr>
<tr>
<td></td>
<td>No.6 village</td>
<td>06</td>
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<tr>
<td></td>
<td>No.7 village</td>
<td>07</td>
</tr>
<tr>
<td></td>
<td>No.8 village</td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>No.9 village</td>
<td>09</td>
</tr>
</tbody>
</table>

(5) General Coding Rule:

Province Code + Urban/Rural Code + City/County Code + City Committee/Village Code
(Coding table for provinces: see annex 13);

2.3.2 Coding volunteering individuals

The survey involves nine provinces, covering two hundred survey sites, and each includes 20-30 volunteering individuals. Each site is expected to follow the previous coding rules and fill in the Basic Information Verification Form.

2.3.3 Barcode ID for volunteering individuals

2.3.3.1 The first and second line of barcode implies its usage. Specifically, "KIT" stands for supplies packaging, "SERUM" for serum samples collection supplies, "SERUM # 0" for vacuum blood collection tubes of serum, "SERUM # 1 # 2 # 3 # 4" for cryovials containing serum; "EDTA" on behalf of EDTA anticoagulation samples collection supplies, "PLASMA # 1" for the cryovial
containing plasma, "RBC # 2" for the cryovial containing red blood cells, "BUFFY COAT # 3" for the cryovial containing the buffy coat, "WHOLE BLOOD # 4" for the cryovial containing whole blood; "BLOOD SPOTS" for blood spots card; "TOENAILS" for toenails collection envelopes; "China-Japan Friendship Hospital, Ministry of Health" is the abbreviation of the China-Japan Friendship Hospital, applied in questionnaires, rosters and all other documents.

2.3.3.2 Numbers and letters of the third row represent the ID code of the sample. In "9001ES0", for example, "9001" is interpreted as ID for the volunteering individual. "E" represents province, corresponding to the upper right corner of the bar code number "41" (see table below). "S" on behalf of sample type: S stands for serum or SST vacuum vessel, E for EDTA vacuum vessel, B for blood point card, T for toenails. "0" represents the sample number, with 0 be the original sample.

<table>
<thead>
<tr>
<th>Province</th>
<th>Letter code</th>
<th>Number code</th>
<th>interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liao Ning</td>
<td>A</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Hei Long Jiang</td>
<td>B</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Jiang Su</td>
<td>C</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Shan Dong</td>
<td>D</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>He Nan</td>
<td>E</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Hu Bei</td>
<td>F</td>
<td>42</td>
<td></td>
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<tr>
<td>Hu Nan</td>
<td>G</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>Guang Xi</td>
<td>H</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Gui Zhou</td>
<td>I</td>
<td>52</td>
<td></td>
</tr>
</tbody>
</table>

2.3.4 Unique Code for each volunteering individual

Ethics of the U.S. population survey requires that survey questionnaire and dietary sampling of one individual should be labeled with different codes. This survey adopts the Unique Code as a connection code between questionnaire and diet sample. Therefore, correctly labeling the Unique Code is crucial in this survey. This coding step is completed in the TO variables of blood sample collection questionnaire and toenail collection questionnaire.

2.3.5 Coding rules for staff and laboratories

To ensure the quality and traceability of samples, each province is required to code its own staff and laboratories according to the rules represents in the table below. Coding forms need to be returned to the project group of China-Japan Friendship Hospital.

<table>
<thead>
<tr>
<th>province</th>
<th>Letter code</th>
<th>Number code</th>
<th>Staff code</th>
<th>Lab code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liao Ning</td>
<td>A</td>
<td>21</td>
<td>101-160</td>
<td>180-199</td>
</tr>
<tr>
<td>Hei Long Jiang</td>
<td>B</td>
<td>23</td>
<td>201-260</td>
<td>280-299</td>
</tr>
<tr>
<td>Jiang Su</td>
<td>C</td>
<td>32</td>
<td>301-360</td>
<td>380-399</td>
</tr>
<tr>
<td>Shan Dong</td>
<td>D</td>
<td>37</td>
<td>401-360</td>
<td>480-499</td>
</tr>
<tr>
<td>He Nan</td>
<td>E</td>
<td>41</td>
<td>501-560</td>
<td>580-599</td>
</tr>
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<td>Hu Bei</td>
<td>F</td>
<td>42</td>
<td>601-660</td>
<td>680-699</td>
</tr>
</tbody>
</table>
2.4 Specimen Collection

2.4.1 Informed consent

Inform all volunteering individuals of purpose of the survey, its methods and responsibilities, and their rights and obligations.

2.4.2 Questionnaires

2.4.2.1 Blood sample questionnaire: record basic information about individuals involving in blood samples collection.

2.4.2.2 Toenails collection questionnaire: record basic information on toenails sample collections.

2.4.3 Specimen Collection

2.4.3.1 Blood samples: Collect three tubes (one 4ml EDTA anticoagulant, two 4ml with separating gel), a total of 12ml of blood for individuals greater than seven years old (seven included).

2.4.3.2 Toenail samples: Collect 10 pieces of toenails (each from one toe) for individuals greater than two years (two included).

2.4.4 Sample Test (see table below):

2.4.4.1 On-site testing: complete within local laboratories of each site, includes the routine blood test (hematology instrument using three categories above; * indicates must include item) and other biochemical tests.

2.4.4.2 Provincial laboratory testing: testing of glycosylated hemoglobin (HbA1c) needs to be completed only in one of the provincial laboratories, which meets all requirements for accurate measurement and testing.

2.4.4.3 Beijing Central Laboratory testing: testing of 20 biochemical and immunological detection is to be completed in China-Japan Friendship Hospital, Ministry of Health laboratory.

<table>
<thead>
<tr>
<th>On-site testing items</th>
<th>Testing items in Beijing Central Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine Blood Test</td>
<td>Biochemical Tests</td>
</tr>
<tr>
<td>* WBC 白细胞数</td>
<td>Glu 血糖</td>
</tr>
<tr>
<td>* RBC 红细胞数</td>
<td>TC 总胆固醇</td>
</tr>
<tr>
<td>* HGB 血红蛋白</td>
<td>HDL-C 高密度脂蛋白胆固醇</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>* PLT</td>
<td>血小板数</td>
</tr>
<tr>
<td>LYM</td>
<td>淋巴细胞总数</td>
</tr>
<tr>
<td>MID</td>
<td>中间细胞总数</td>
</tr>
<tr>
<td>GRAN</td>
<td>中性粒细胞总数</td>
</tr>
<tr>
<td>HCT</td>
<td>红细胞比积</td>
</tr>
<tr>
<td>MCV</td>
<td>红细胞平均体积</td>
</tr>
<tr>
<td>MCH</td>
<td>平均血红蛋白含量</td>
</tr>
<tr>
<td>MCHC</td>
<td>平均血红蛋白浓度</td>
</tr>
<tr>
<td>RDW</td>
<td>红细胞分布宽度</td>
</tr>
<tr>
<td>MPV</td>
<td>平均血小板体积</td>
</tr>
<tr>
<td>PDW</td>
<td>血小板分布宽度</td>
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<tr>
<td>LYM%</td>
<td>淋巴细胞百分数</td>
</tr>
<tr>
<td>MID%</td>
<td>中间细胞百分数</td>
</tr>
<tr>
<td>GRAN%</td>
<td>中性粒细胞百分数</td>
</tr>
<tr>
<td>PCT</td>
<td>血小板压积</td>
</tr>
<tr>
<td>Glu</td>
<td>葡萄糖</td>
</tr>
<tr>
<td>ALT</td>
<td>丙氨酸氨基转移酶</td>
</tr>
<tr>
<td>TP</td>
<td>总蛋白</td>
</tr>
<tr>
<td>ALB</td>
<td>白蛋白</td>
</tr>
<tr>
<td>TC</td>
<td>总胆固醇</td>
</tr>
<tr>
<td>TG</td>
<td>甘油三酯</td>
</tr>
</tbody>
</table>

* Required items in Routine blood test
Specimen Collection Flow Chart

- Preliminary Preparation:
  - Preparation of specimen collection supplies, equipment and documents
  - Place barcode labels as required
  - Be familiar with collection procedure, testing quality control samples

- In-home Notification I:
  - (Verify basic information of involved individuals)
  - Issuing and collection of informed consent
  - Issuing notice of venipuncture
  - Issuing materials for toenails collection

- In-home notification II:
  - Reminding date for blood sample collection
  - Stress procedures and precautions for blood sample collection
  - collect toenail samples

- Blood samples collection day:
  - Blood samples collection
  - Sample packing

- Blood samples collection:
  - Sample preservation

Survey Summary:
  - Sorting original data
  - Checking blood survey questionnaires
  - On-site test results feedback

- Sample packing:
  - Ship to Provincial Laboratory for preservation

- Sample preservation:
  - Ship to Beijing Central Laboratory for preservation
## Working schedule

<table>
<thead>
<tr>
<th>Day</th>
<th>Morning (8:00-11:30)</th>
<th>Afternoon (2:00-5:00)</th>
<th>Evening (7:00-9:00)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Co-operators of Chinese-Japan Friendship Hospital</td>
<td>CDC and its co-operators</td>
<td>Co-operators of Chinese-Japan Friendship Hospital</td>
</tr>
<tr>
<td></td>
<td>Technicians training and supplies preparation prior to sample collection</td>
<td>Issuing and collection of informed consent</td>
<td>In-home nutritional measurement</td>
</tr>
<tr>
<td></td>
<td>In-home questionnaires; GPS data collection of community residence</td>
<td>Issuing notice of venipuncture</td>
<td></td>
</tr>
<tr>
<td></td>
<td>In-home questionnaires; GPS data collection of other community spots</td>
<td>Issuing materials for toenails collection</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Reminding date and precautions of venipuncture; toenails sample collection</td>
<td>Complete all questionnaires; remind volunteers of venipuncture</td>
<td></td>
</tr>
<tr>
<td></td>
<td>On-site blood sample test and preservation</td>
<td>Health status examination</td>
<td>Specimen ship to local or provincial laboratory for centrifugation; checking questionnaires and conducting on-site summary; health status feedback</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Third day of nutritional investigation and measurement</td>
</tr>
<tr>
<td>3</td>
<td>Reminding date and precautions of venipuncture; toenails sample collection</td>
<td>Complete all questionnaires; remind volunteers of venipuncture</td>
<td></td>
</tr>
<tr>
<td></td>
<td>On-site blood sample test and preservation</td>
<td>Health status examination</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Supplemental sample collection</td>
<td>Questionnaires verification and correction</td>
<td>The end of pre-investigation</td>
<td></td>
</tr>
</tbody>
</table>
3 PREPARATIONS

Since participation in this survey is voluntary, every effort must be made to make the entire procedure as easy and painless as possible for participants. Technicians must remain calm and project an attitude of competence even when faced with the most nervous or inquiring participant. The best way to achieve this is for the technicians to be thoroughly knowledgeable about all aspects of the procedures.

3.1 Staff Certification Requirements

Blood drawing and processing are performed by certified technicians at each field center. The technicians complete a training course taught by certified laboratory staff. Each technician must complete the training and pass all exams before becoming certified.

3.2 Equipment and Supplies

One day prior to a scheduled participant visit, the technician prepares two trays: one to hold the blood collection tubes, another to hold the plastic vials which will hold the final packed cells, serum, plasma, and urine aliquots until they are frozen and ultimately transferred to the Central Laboratories for analysis. Most of the supplies are provided by China-Japan Friendship Hospital, while each site is expected to prepare the remaining parts by their own. Technicians need to be familiar with the properties and usage for all kinds of supplies before the project starts. A list of equipment, suppliers, and vendors is provided in the chart below:

Electronic versions of forms and questionnaires will be provided by China-Japan Friendship Hospital, and each site is expected to print according to their practical needs.

<table>
<thead>
<tr>
<th>Type</th>
<th>Name</th>
<th>Role in the survey</th>
<th>Responsible agent(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>labels</td>
<td>Bar code labels</td>
<td>Placed on the documentation of various blood supplies and for coding purpose</td>
<td>University of North Carolina</td>
</tr>
<tr>
<td>Documents and questionnaires</td>
<td>Document forms</td>
<td>Control the quality of work</td>
<td>China-Japan Friendship Hospital(electronic versions)</td>
</tr>
<tr>
<td></td>
<td>Informed consent</td>
<td>Inform volunteering individuals of their duties and obligations</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blood sample questionnaires</td>
<td>Collect information related to blood samples collection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Toenails sample questionnaires</td>
<td>Collect information related to toenails samples collection</td>
<td></td>
</tr>
<tr>
<td>Blood sample collection</td>
<td>Test tube racks</td>
<td>On-site blood sample collection and temporary storage</td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>----------------</td>
<td>------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>tourniquet, blood collection supplies (sterile alcohol swabs)</td>
<td>Blood collection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trash bag (medical purpose)</td>
<td>Disposal and collection of medical waste</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sterile, disposable 21 gauge butterfly needles</td>
<td>Match with vacuum blood collection tubes during blood sample collection process</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4ml lavender-stoppered EDTA vacuum blood collection tube</td>
<td>Collects whole blood for HbA1c determination; Separates plasma, buffy coat, and red blood cell by centrifugation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4ml red-stoppered(with yellow ring) vacuum blood collection tube with separating gel</td>
<td>Centrifugal separation of plasma for the determination of biomarkers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood constituents separation</td>
<td>blue-stoppered cryovial</td>
<td>Contains EDTA anticoagulant for whole blood storage</td>
<td></td>
</tr>
<tr>
<td>green-stoppered cryovial</td>
<td>Contains EDTA anticoagulant for plasma storage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>white-stoppered cryovial</td>
<td>Contains EDTA anticoagulant for buffy coat storage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>red-stoppered cryovial</td>
<td>Contains EDTA anticoagulant for red blood cell storage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>yellow-stoppered cryovial</td>
<td>serum storage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cryovial storage boxes</td>
<td>Store different kinds of cryovials</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disposable pipette</td>
<td>Separate different blood constituents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood spot card</td>
<td>Collect blood spots</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood processing and shipping</td>
<td>reefer</td>
<td>Immediate and transitional storage</td>
<td></td>
</tr>
<tr>
<td>Dry ice</td>
<td>Immediate and transitional storage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood preservation</td>
<td>2-8℃ refrigerator</td>
<td>Samples preservation</td>
<td></td>
</tr>
<tr>
<td>-70℃ freezer</td>
<td>Samples preservation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toenails collection</td>
<td>Toenail scissors</td>
<td>For toenail collection</td>
<td></td>
</tr>
</tbody>
</table>

**3.2.1 Barcode**
For the purpose of future management and data applications, bar code labeling is adopted in this survey. Unified ID will be labeled on the questionnaires, vacuum blood collection tubes and cryovials. Technicians need to prepare in advance a plastic bag with necessary supplies, with corresponding barcodes labeled, for each volunteering individual.

### 3.2.2 Forms
See Appendix. For specific applications, see the contents of this manual.

### 3.2.3 Lavender-stoppered EDTA vacuum blood collection tube

(1) EDTA K3 attachments of the inner tube walls and rotary screw-type cover can prevent generation of aerosol and ensure transformational safety
(2) In order to obtain reliable test results, centrifugation of the blood sample should be performed within 3 hours
(3) recommended storage temperature of the sample:
   - Short-term: -2 ~ 8 ℃;
   - Long-term: stored in individual cryovial, -70 ℃.

### 3.2.4 Red-stoppered (with yellow ring) vacuum blood collection tube with separating gel

(1) Separation gel at the bottom of tube has a density between density of blood clot and serum, forming a stable boundary layer that isolates serum from fibrin and blood cells.
(2) Silicon particles attached to the inner wall surface can result in blood coagulation when gently reverse the tube;
(3) Centrifugation should be performed **30 MINUTES AFTER** blood collection, with 3000g, 15 minutes;
(4) In order to obtain reliable test results, centrifugation of blood sample should be performed within 3 hours.
NOTE: to reduce damage caused by vibration, blood collection tubes should be placed upright at room temperature for at least one hour after centrifugation before further transportation.

3.2.5 Cryovials

Cryovials are composed by 1.8ml polypropylene plastic material (11mm × 45mm). Each cryovial contains 0.5-1.0ml samples of different components, and has one of the five different color-coded screw caps. All cryovials have no biological activity, present high resistance to oxidation, and can withstand temperatures below -70 ℃. These cryovials can.

3.2.6 Cryovial storage boxes

9 × 9 cryovial storage boxes are made by paper (105mm × 105mm), matching 1.8ml cryovials. These boxes are used for storage of different components after centrifugation. Boxes are labeled 1 to 9, A to I for records.

3.2.7 Blood spot card

Specially crafted blood spot cards by Whatman 903 filter paper are adopted for the collection of EDTA whole blood. Drop blood sample with a disposable pipette. Let the blood sample dry for 30 minutes, and then get it back into its supply bag. Adhesive tape is needed for hinge. Blood spot cards used in this survey are 5 points / per card / per person (see figure below).
3.2.8 Disposable pipette

With a scale of 0.25ml per unit and a maximum absorption capacity of 1ml, the pipettes are used for blood dripping and components separation.

3.3 Labeling Process

For the purpose of future sample management and data application, bar code technology is applied in this survey. Questionnaires, vacuum blood collection tubes and cryovials will have bar code labeled uniformly. Technicians need to prepare each volunteer a plastic bag of all necessary supplies, with corresponding bar code affixed.

3.3.1 Labeling Process

In understanding all the rules and use, Bar coding process should be strictly manipulated according to the following procedures, and labeling of related supplies should be completed before all work starts.

3.3.1.1. Pre-numbered barcodes

Pre-numbered barcodes refers to the bar coding process prior to sample collection, which should be completed during the preparation period before all work starts.

(1) preparing one supply bag, one EDTA vacuum blood collection tube , two vacuum blood
collection tubes with separating gel, cryovials with yellow, blue, green, and white tops (four each), and one blood spots card;
(2) First apply pre-numbered barcode laboratory ID labels to each supply bag on its upper left corner;
(3) Apply the barcode labels to three vacuum blood collection tubes as shown in the figure below;
(4) Apply the barcode labels to eight different cryovials as shown in the figure bellow;
(5) Apply the barcode labels to the blood spot card.

3.3.1.2 On-site bar coding process

Upon completion of the previously described preparation, 13 barcode labels should have been used. Among the remaining seven barcode labels, one will be applied to an envelope for toenails collection, one on Basic Information Verification Form, and three on Blood Sample Collection Questionnaires.

For detailed procedures, please see BLOOD SAMPLE COLLECTION of this manual.

3.3.1.3 Extra barcode labels

All extra labels should be put in the supply bag and returned to the Central Laboratory (China-Japan Friendship Hospital).

3.3.2 Barcode labels
3.3.3 Barcode labels Table

<table>
<thead>
<tr>
<th>Pre-numbered barcode labels (13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supply bag</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Lavender-stoppered EDTA vacuum blood collection tube</td>
</tr>
<tr>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td>Green-stoppered cryovial</td>
</tr>
<tr>
<td>Red-stoppered cryovial</td>
</tr>
<tr>
<td>White-stoppered cryovial</td>
</tr>
<tr>
<td>Blue-stoppered cryovial</td>
</tr>
</tbody>
</table>

**Remaining barcode labels (7)**

**On-site barcode labels (5)**

<table>
<thead>
<tr>
<th>First part of the blood collection questionnaire</th>
<th>Basic Information Verification Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second part of the blood collection questionnaire</td>
<td>Toenails collection</td>
</tr>
<tr>
<td>Third part of the blood collection questionnaire</td>
<td></td>
</tr>
</tbody>
</table>

**Remaining barcode labels (2)**

### 3.3.4 Precautions in the labeling process

1. Labeling the blood collection tube:

![Barcode Label Example](image-url)
A. Place the label vertically on the tube, with the bar-code oriented from the bottom of the tube to the top of the tube (for easy scanning);
B. Labels should be placed below the original ones, leaving space for serial number markings and name tags;
C. Labels should not cover the transparent part of the tubes.

(2) Labeling cryovials:

A. Place the label vertically on the tube, with the bar-code oriented from the bottom of the tube to the top of the tube (for easy scanning);
B. Labels should not cover the scale.
C. Check solidity after labeling

(3) Second party verification is required to minimize the chance of mislabeling.

### 3.4 Preparation of Volunteer name list

**3.4.1** Since the survey is completed by different investigation groups, each group will receive a list of all volunteers from 1989 to 2006 (see table below). Therefore, not all individuals on the list can be covered in 2009, and only those who would participate are our primary objects in this specimen collection process. The following table provides the basic information of qualified individuals.

<table>
<thead>
<tr>
<th>Only Code</th>
<th>Name</th>
<th>Participate in 2009?</th>
<th>Gender</th>
<th>Date of Birth</th>
<th>Date of Birth (in Lunar calendar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10001</td>
<td>Bingcheng Huang</td>
<td>1</td>
<td>1</td>
<td>19550203</td>
<td>19550111</td>
</tr>
<tr>
<td>10002</td>
<td>Jinhui Huang</td>
<td>0</td>
<td>1</td>
<td>19811229</td>
<td>19811204</td>
</tr>
</tbody>
</table>

**3.4.2** Joint team of specimen collection group and Chinese Center for Disease Control and Prevention nutritional investigation group should check the basic information of volunteering
individuals with the name list, and completing the Basic Information Verification Form in Appendix 3. For example, No.10001 is participating in the survey of 2009, and so his information should be recorded by filling out the Basic Information Verification Form. There is no need to record basic information of No.10002 since he is not participating in the survey.

<table>
<thead>
<tr>
<th>Only Code</th>
<th>Name</th>
<th>Gender</th>
<th>Date of Birth</th>
<th>Date of Birth (in Lunar calendar)</th>
<th>Family Code</th>
<th>Home Address</th>
<th>Barcode</th>
</tr>
</thead>
<tbody>
<tr>
<td>10001</td>
<td>Bingcheng Huang</td>
<td>1</td>
<td>19550203</td>
<td>19550111</td>
<td>01</td>
<td></td>
<td>XXXX</td>
</tr>
</tbody>
</table>

3.4.3 On the blood sample collection day, technician should first verify the basic information of the volunteer before attaching bar code sticker to its Basic Information Verification Form.

<table>
<thead>
<tr>
<th>Only code</th>
<th>Name</th>
<th>Gender</th>
<th>Date of Birth</th>
<th>Date of Birth (in Lunar calendar)</th>
<th>Family Code</th>
<th>Home address</th>
<th>barcode</th>
</tr>
</thead>
<tbody>
<tr>
<td>10001</td>
<td>Bingcheng Huang</td>
<td>1</td>
<td>19550203</td>
<td>19550111</td>
<td>01</td>
<td></td>
<td>XXXX</td>
</tr>
</tbody>
</table>

3.5 Informed Consent

3.5.1 Informed consent specifies duties and obligations of participants, and volunteers must sign before participating in the survey;

3.5.2 Investigators should contact all volunteers and explain the informed consent prior to in-home visits. This informed consent is better being signed together with the CDC one, and investigators need to explain all benefits to the volunteers such as free health status examination and free blood test feedback.

3.5.3 Informed consent should be signed directly by the volunteer; if the volunteer is unable to provide valid signature, qualified guardian or family members may be considered for signature.

3.5.4 Signature of the investigator is required upon completion of the informed consent.

3.6 Requirements of Questionnaires and Forms

3.6.1 Investigators must be familiar with the questionnaire and protect the privacy right of volunteers. Investigators can provide appropriate explanation if the volunteer encounter understanding
difficulties. Investigators should ask questions in order to minimize the possibility of recording errors.

3.6.2 Investigators are responsible for verification all information presented on the questionnaires. Logical errors, typos, and other errors should be identified and corrected in this process. Major verification items include: name, personal ID code, date of birth and other basic information.

3.6.3 All questionnaires need to be completed by pen, with clear writing (in standard form) and no correction. "0" should not be omitted; for example, for the time 6:30 am, $6\colon30$ is the right way of recording instead of $6\colon3\ 0$.

3.6.4 If any error is identified in the submitted questionnaire, follow these steps for effective correction: first double strikethrough the wrong character or number, and then write down the correct terms on its top. For instance, the right way to change 6:30 to 6:35 is:

$$0 6:30 \rightarrow 0 6:35$$

3.6.5 **Blood sample collection questionnaire**

Blood sample collection questionnaire plays a vital role in quality control of samples. Questionnaires should be carefully filled out before the start of the sample collection process. General information included in the first part of the questionnaire need also be checked (name, code, date of birth, gender, etc.).

Detailed procedure:

(1) Investigator A fill out the questions 1-7 in Part 1 of the questionnaire according to the *Basic Information Verification Form* (Appendix 3). Be sure to fill in the correct Unique Code;

(2) Investigators A tries to complete questions 8-12 of Part 1 (not measured, but important in practice).

(3) Complete questions 13-23 in Part 1 by consulting the volunteers.

(4) Investigators B and C record and fill out questions 1-3 in Part 2 of the questionnaire after sample collection;

(5) Technicians D and E are responsible for completing questions 4-11 in Part 2 and all questions in part 4;

(6) **Staff and laboratory coding rules**

<table>
<thead>
<tr>
<th>Provinces</th>
<th>Letter Code</th>
<th>Number Code</th>
<th>Staff Code</th>
<th>Laboratory Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liao Ning</td>
<td>A</td>
<td>21</td>
<td>101-160</td>
<td>180-199</td>
</tr>
</tbody>
</table>
### 3.6.6 Toenail collection questionnaires

Name and code of each volunteering individual should be labeled before dispersion of the questionnaires. Investigators are also responsible for guiding volunteers to complete the questionnaires, as well as checking completion of the questionnaires.

### 3.6.7 Quality Control of the Questionnaires

(1) All investigators should be trained about standard rules in completing the questionnaires;

(2) Basic information deserve carefully check to ensure that the questionnaire corresponds its sample;

(3) During random inspection conducted by the supervisor, the completion rate should reach 90%, and the qualification rate should reach 95%; percentage of pass in the verification inspection should be 100%;

### 3.6.8 Appendix 1 - Samples Transfer Record Sheet

(1) Record the entire transferring and shipping process for each batch of samples. A separate sheet of form is required for each batch even if only one sample is included;

(2) Type and quantity of samples, time and temperature of the sample transfer, transit and receiving staff signature must all be indicated in the form;

(3) Each form is expected to be clearly labeled its sample source and community code.

### 3.6.9 Appendix 2 - On-site Laboratory Information Form

(1) Record basic information of on-site labs to facilitate further application of laboratory results from provincial labs;

(2) Name of the on-site lab and equipment must be indicated in the form.

(3) Signatures of both parties, persons who fill out the form and who are responsible to the form, are
required.

3.6.10 Appendix 3 - Basic Information Verification Form

(1) Verify basic information of volunteers to facilitate the conduct of further investigation;

(2) for detailed rules, see 3.4 Preparation of Volunteer name list of this manual.

3.6.11 Appendix 4 - Samples Preservation Temperature Record Sheet

(1) Record temperature control of the samples. If no environmental change occurs, record should be taken once a day.

(2) Type, quantity, storage temperature, and storage equipment of the samples should be recorded clearly and signed;

(3) Each form is expected to be clearly labeled its sample source and community code.

3.6.12 Appendix 5 - Samples Preservation Information Form

(1) Record the basic information of processed samples;

(2) Type, quantity, and bar code of the sample must be recorded in the form;

(3) For detailed rules, see 6.1.4 Storage of cryovials of this manual;

(4) This form should contain the same information as shown on the frozen pipes.

3.6.13 Appendix 6 - On-site Collection Summary Sheet

(1) Summarization of on-site sample collection;

(2) Completion of each part of the work must be thoroughly recorded;

(3) State specific reasons to those work that cannot completed.

3.6.14 Appendix 12 - Routine Internal QC Plan

Record the internal quality control of the on-site lab one month prior to sample collection. This record is considered part of the survey’s quality control, which checks the preciseness and accuracy of measurement of the lab.
4 BLOOD SAMPLE COLLECTION

Blood samples are one of the most important specimens in this survey. All individuals older than seven years old (seven years old included) are required to be collected 12ml blood (in three 4ml tubes) on empty stomach. In order to ensure that all further tests would be conducted smoothly, collection and processing of blood samples must stick strictly to the rule.

4.1 Blood Sample Collection Flow Chart
4.2 On-site Blood Collection List

Supplies listed in the table below should be prepared before the start of blood sample collection (for 100 people).

Note: blood collection supplies and documents are provided by China Japan Friendship Hospital, and all other materials need to be prepared by local co-operators, including first-aid kits and emergency items.

<table>
<thead>
<tr>
<th>On-site supplies</th>
<th>Blood collection supplies (100)</th>
<th>Documents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>Quantity</td>
<td>Item</td>
</tr>
<tr>
<td>Document station (desk)</td>
<td>1</td>
<td>Blood spots card</td>
</tr>
<tr>
<td>Venipuncture station (desk)</td>
<td>1</td>
<td>4ml lavender-stoppered EDTA vacuum blood collection tube</td>
</tr>
<tr>
<td>chair</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Table cover</td>
<td>2</td>
<td>4ml red-stoppered (with yellow ring) vacuum blood collection tube</td>
</tr>
<tr>
<td>Test tube racks</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Arm holster</td>
<td>2</td>
<td>Blue-stoppered cryovial</td>
</tr>
<tr>
<td>Tourniquets</td>
<td>2</td>
<td>Green-stoppered cryovial</td>
</tr>
<tr>
<td>Cotton swab</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>tray</td>
<td>2</td>
<td>White-stoppered cryovial</td>
</tr>
<tr>
<td>Waste bin (medical)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Waste bags (medical)</td>
<td>10</td>
<td>Red-stoppered cryovial</td>
</tr>
<tr>
<td>sockets</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>On-site centrifugation</td>
<td>1</td>
<td>Yellow-stoppered cryovial</td>
</tr>
<tr>
<td>Disposable gauge</td>
<td>110</td>
<td>Disposable pipette</td>
</tr>
</tbody>
</table>
4.3 Division of Duties and Work Flow

Specimen collection team should include at least five people: one investigator (A), two blood collection technicians (B and C), and two inspectors (D and E), specific work flow is as follows:

4.3.1 The investigator (A) is responsible for coordination with the community and liaison with local CDC; the first in-home notification, distribution of Toenails Collection Form (Appendix 11) and envelopes; organize the second in-home notification and collect the toenail collection form on blood sample collection day; complete questions 1-23 in Part 1, general information, of the Blood Sample Collection Questionnaire (Appendix 10);

4.3.2 Blood collection technicians (B and C) are responsible for verifying information of the volunteer according to the Blood Sample Collection Questionnaire; place the appropriate bar codes labels (three) onto the Blood Sample Collection Questionnaire; blood sample collection; complete the questions 1-3 in Part 2 (Sample Collection) of the questionnaire;

4.3.3 Inspectors (D and E) are responsible for checking numbers and quantities of vacuum blood collection tubes (one 4ml tube containing EDTA anticoagulant, two 4ml tubes containing blood separation gel), blood sample processing, on-site determination, preservation and transportation. (For detailed test method, see below). Lab staff should follow the principle of “individuals are responsible for their own work, while coordination is also expected among staff.”

4.4 Participants Preparation

4.4.1 Maintain a regular pattern of life for at least three days before blood sample collection;

4.4.2 No food intake 8-12 hours prior to the blood sample collection;

4.4.3 Relaxation during blood drawing may help avoid vessel contraction caused by inner fear;

4.4.4 Wear loose clothing before and during blood drawing;

4.4.5 In order to avoid partial hematoma, push hard at the wounded spot with swabs for 3-5 minutes
immediately after blood drawing. Then throw swabs into the medical waste bucket.

4.4.6 Avoid shower or bath the day of blood drawing for wound healing purpose;

4.4.7 If suspicious symptoms occur, such as dizziness, nausea, palpitation, and cold sweats, report to the doctors immediately, or sit for a while before symptoms relief;

4.4.8 Avoid food intake before completing the whole medical examination.

4.5 Venipuncture

4.5.1 Preparation of vacuum blood collection supplies

a. Wash hands and put on gloves;
b. Assemble the necessary equipment: test tube rack, blood pillow, butterfly blood collection needles, cotton swabs, etc.
c. Guide the volunteer to sit in front of the blood collection stage, collect their forms and verify information presented on the form.
d. Briefly explained the procedures of blood drawing. Reassure the patient that the minimum amount of blood required for testing will be drawn.
e. Position the patient with the arm extended to form a straight-line form shoulder to wrist.

Vacuum blood collection system: provided by Austrian VACUETTE vacuum blood collection system

4.5.2 Selecting appropriate vein for venipuncture

The larger median cubital, basilic and cephalic veins are most frequently used, but other may be necessary and will become more prominent if the patient closes his fist tightly. Apply the tourniquet 3-4 inches above the collection site. Clean the puncture site by making a smooth circular pass over the site with the 70% alcohol pad, moving in an outward spiral from the zone of penetration. Allow the skin to dry before proceeding. Do not touch the puncture site after cleaning.
4.5.3 Preparation of butterfly blood needle

Hold the butterfly blood needle and perform the venipuncture. If blood starts to flow, which marks successful venipuncture, then slightly push the evacuated tube. Blood should flow into the tube by pressure difference.

4.5.4 Venipuncture

Pull the skin tight with index finger of right hand just below the puncture site. Holding the needle in an angle of 15° with the vein, then use a quick, small thrust to penetrate the skin and enter the vein in one smooth motion.
4.5.5 Tourniquet

Never leave the tourniquet on for over 2 minutes. If a tourniquet is used for preliminary vein selection, release it and reapply after two minutes. After blood starts to flow, release the tourniquet and ask the patient to open his or her hand.

4.5.6 Blending

When blood flow stops, remove the tube by holding the hub securely and pulling the tube off the needle. Holding the hub securely, insert another tube following the same procedure. Each tube containing an additive should be gently inverted 5-8 times after being removed.

NOTE: NEEDLES MUST BE KEPT IN PLACE AT ALL TIMES.

4.5.7 Prevention of bleeding

Place a gauze pad over the puncture site and remove the needle. Immediately apply slight pressure. Ask the patient to apply pressure for at least 2 minutes. Properly dispose of hub with needle
attached into a sharps container.

4.6 Precautions

4.6.1 If the participant is nervous or excited, the technician should briefly describe the procedure. If the participant is very anxious, he/she may lie down during the blood collection. When a Participant feels/Looks faint Following the Blood Drawing: Have the person remain in the chair. If necessary, have him/her lie on the floor with legs elevated. Use of a transfer belt may be indicated in this situation. Take an ampule of smelling salts, crush it, and wave it under the person's nose for a few seconds. Once the episode has passed, some fruit juice may be given to the participant in order to counteract any possible hypoglycemia due to their pre-clinic visit fast. If the person continues to feel sick, take a blood pressure and pulse reading. Contact a medical staff member for further direction.

4.6.2 Hemolysis should be prevented. Several factors that result in hemolysis: wet or un-cleaned equipment; long tourniquet time that cause congestion; excessive injured tissues caused by unsuccessful venipuncture; bubbles generated by improper removing of needles; vigorous oscillation of anticoagulant agents; high speed of centrifugation.

4.6.3 The tourniquet should be on the arm for the shortest time possible. Never leave the tourniquet on for longer than half minute. To do so may result in hemoconcentration or a variation in blood test values.

4.6.4 If vein collapse during venipuncture, all operations should stop immediately and try the other arm. If it occurs again, venipuncture of the volunteer should be stopped and briefly record the reasons on the form.

4.6.5 If the first venipuncture fail to collect enough blood, or the collection tube cannot collect blood automatically, extra collection tube should be adopted immediately, with barcode labels affixed.

4.7 Emergency Measurements

4.7.1 Fainting (syncope)

Causation: Some people felt dizzy or even faint upon seeing blood.

Solution: Technicians should ask about the volunteer’s previous venipuncture experience, and venipuncture should be performed in lying position. If the volunteer faints during the venipuncture process, technicians should immediately stop and check if any injury occurs.

4.7.2 Hematoma

Causation: A hematoma is caused when blood leaks around the puncture site into the tissues. This can occur when the needle penetrates completely through the vein or improperly accesses the vein
Solution: If a hematoma begins to form, quickly remove the tourniquet and needle, and then apply pressure for approximately 2 minutes. A patient will normally stop bleeding at the venipuncture site after a few minutes unless he's on anticoagulant therapy.

4.7.3 Bruise

Causation: small red spots appear on the skin, caused by a small amount of blood that ran into the upper epidermis.

Solution: patients in such situation may have experienced excessive bleeding. Make sure bleeding stops before the patient leaves after the venipuncture.

4.7.4 Obesity

Causation: It is generally more difficult to identify proper veins for venipuncture in individuals suffering obesity.

Solution: spend more time finding a suitable vein but avoid excessive attempts.

4.7.5 Allergy

Causation: Some participants are allergic to iodine or fluorine.

Solution: Try to find alternatives if such symptom occurs.

4.8 Quality Control in Blood Sample Collection

4.8.1 Hemolysis

Causation: the relatively high pressure in vacuum tube leads to a high speed of blood infusion; collision among red blood cells causes hemolysis.

Solution: tilting the needle that allows blood to shed slowly along the wall, which helps avoid rupture of red blood cells caused by collision.

4.8.2 Blood leakage

Causation: loose latex sheath enables slight leakage of blood during venipuncture.

Solution: detect possible leakage prior to the venipuncture process..

4.8.3 Poor blood flow
Causation: puncture needle is tight to the vessel wall or no pressure inside the collection tube.

Solution: Adjust the direction of the needle until successive blood flow into the tube. Otherwise, replace the collection tube.

4.8.4 Inadequate or excessive blood

Causation: lack or excessive pressure inside collection tube.

Solution: (1) Replace the collection tube directly. (2) Pull out the needle earlier.

4.8.5 Prolapses of the puncture needle

Causation: mechanical stress, especially when collecting blood by many tubes.

Solution: Effectively fix the tube and avoid large motion that may potentially draw the needle out.

5 TOENAIL SAMPLES COLLECTION

5.1 Target Population

Volunteering individuals older than 2 years old (2 years old included).

5.2 Requirements of Collection

In order to obtain more samples of toenails, volunteers are asked to keep toenails growing for two weeks before using stainless steel nail scissors to collect their toenails as more as possible. The toenail samples can be placed in the labeled envelope and kept in try condition at room temperature. There’s no need to remove nail enamel.

5.3 Collection Procedure

5.3.1 Disperse toenail collection questionnaire, envelope, and toe nail scissors among volunteers.
5.3.2 Ask the volunteers to collect toe nails of 10 toes and place them in the envelope.

5.3.3 Ask the volunteers to answer the four questions in the questionnaire (Appendix 11);

5.3.4 Toenails collection should be turned into the investigators prior to blood sample collection. Investigators should first verify name and questionnaire before store the samples in dry place;

5.3.5 After completion of sample collection at each site, all samples will be sent to the provincial central laboratory for preservation at the same time.

5.3.6 Upon completion of sample collection of the whole province, samples will be sent to Beijing central laboratory for preservation.

5.3.7 Samples sent to Beijing should be placed under dry and dark condition for future analysis.

Note: This survey does not include entry of toenail collection questionnaires.

6 BLOOD SAMPLE PROCESSING AND PRESERVATION

6.1 Blood Sample Processing

6.1.1 On-site processing

6.1.1.1 Investigators (A) get touch with the volunteer at the beginning of the survey and verify information appeared on the Basic Information Verification Form (Appendix 3). Correctly fill in the Only Code is critical in this step;

6.1.1.2 Investigators (A) tries to complete the Part 1 question 8-12 (not measured, but important in practice). Complete Part 1 of the questionnaire and pass it to blood collection technicians (B and C);

6.1.1.3 Blood collection technicians (B and C) need to double check the answers to the questionnaire before conducting specimen collection. Then pick one supply package, and paste bar code stickers
onto each page of the questionnaire;

6.1.1.4 Blood collection technicians (B and C) should collect and label the samples according to rules stated in this manual. When blood sample collection is completed, technicians need to complete Part 2 of the questionnaire, and forward it to inspectors (D and E);

6.1.1.5 Inspectors (D and E) receive the blood samples from the blood collection technicians, and check the name and labeling of vacuum tubes;

6.1.1.6 Inspectors (D and E) are responsible for completing question 4-11 in Part 2 and all questions in Part 3 and Part 4 of the questionnaire. Number IDs of laboratories, blood collection technicians, and inspectors are issued by provincial co-operators, and are expected to be reported to China-Japan Friendship hospital;

6.1.1.7 Inspectors (D and E) are responsible for organizing on-site tests and recording results in the On-site Test Record Form (Appendix 2). (for detailed test method, see Blood Sample Test Manual);

6.1.1.8 Inspectors (D and E) need to store the samples in refer, with a ratio of sample and dry ice to be 1 :1, and transfer them to local laboratory for on-site testing and processing within 3 hours. Inspectors are also expected to complete Samples Preservation Temperature Record Sheet (Appendix 4) and the Samples Transfer Record Sheet (Appendix 1).

6.1.2 EDTA Anticoagulant Blood Processing

6.1.2.1 Preparation of blood spots card

Add one drop EDTA whole blood (50ul) onto the blood spot card with disposable pipette. Dry in room temperature for 30 minutes, and store in the supply bag;

Note: avoid any of the following situations: repeated dropping; touching and smearing the blood spot; too much or too little of the blood spot; scratching the blood spot; the sample is not completely dried; faded or contaminated blood spot.

6.1.2.2 Keep 500ulEDTA whole blood (blue-stoppered cryovial), for on-site blood examination and future HbA1c test;

Note: HbA1c blood can have up to 7 days of storage, so tests need to be conducted as soon as
possible.

6.1.2.3 Centrifugation

Centrifuge the remaining EDTA whole blood sample 3000g for 15minutes;

6.1.2.4 Extraction of Buffy Coat

a Draw plasma with disposable pipette into cryovial (green-stoppered) till 1mm above the buffy coat. Avoid disturbing the buffy coat.

b Carefully draw the buffy coat into cryovial (white-stoppered). Rotated drawing method may be useful in complete buffy coat extraction.

c Store the remaining red blood cells, approximately 300-500ul, into cryovial (red-stoppered).

6.1.2.5 Store cryovials to corresponding boxes.

6.1.3 Blood processing with separating gel

6.1.3.1 Centrifugation: Centrifuge the sample 3000g for 15minutes;

6.1.3.2 Load the serum into cryovials (yellow-stoppered) after centrifugation and store it in cryovial storage box. Ensure the quantity of serum in cryovials #1 and #2, then store the remaining serum in cryovials #3 and #4.

6.1.3.3 #1 will be used in the first laboratory test;  
#2 will be used in the second laboratory test;  
#3 will be used in on-site test;
Note: all numbered cryovials should be sent to Beijing central laboratory for verification and storage.

6.1.3.4 Determine the amount of serum in cryovials based on the amount collected.

<table>
<thead>
<tr>
<th>Amount of serum (ml)</th>
<th>#1 (ml)</th>
<th>#2 (ml)</th>
<th>#3 (ml)</th>
<th>#4 (ml)</th>
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</thead>
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<td>0.5</td>
<td></td>
<td></td>
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<tr>
<td>1.0</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Please verify the bar code labeled on tubes.

6.1.4 Storage of cryovials

6.1.4.1 After all samples are processed in local laboratory, different cryovials should be immediately placed in corresponding frozen storage boxes. Each type and color of cryovials should be stored separately, with a total of 8 cryovials (1 #, 2 #, 3 #, 4 #; 4 yellow top cryovials of serum, 1 blue top cryovial of whole blood, 1 green top cryovial of plasma, 1 white top cryovial of buffy coat, 1 red top cryovial of red blood cells);

6.1.4.2 Attach one sheet of sample storage information form (Appendix 5) in each storage box, including the following information regarding the sample origin: province, city, and district, village, starting and ending series number, sample type, storage date, and technician signature;

6.1.4.3 Fill in the corresponding bar code ID number in the 9X9 square table below; marks “X” if a lack of sample occur. This square table is the information card attached inside the storage box, and a copy of the table should go into a plastic bag inside the storage box along with the sample storage information form.

6.1.4.4 Start with A1, and storage should take place in the order of A1, A2, A3…, B1, B2, B3…, … I1, I2, I3….
6.1.5 Centrifugation

6.1.5.1 See manual for specific laboratory centrifuge operations and balance instruction;

6.1.5.2 Centrifuge for 15 minutes with centrifugal force 3000 × g. Since centrifugal force is proportional to radius of the centrifugal rotor, longer time of centrifugation can make centrifugal force multiplied by time reach 45000 if the on-site centrifuge cannot reach 3000 x g. For example, if the largest centrifugal force is 1500 × g, the centrifugal time should increase from 15 minutes to 30 minutes correspondingly;

6.1.5.3 Cryovials containing separation gel pro-coagulant agents should be placed still for 30 minutes before centrifugation;

6.1.5.4 It is important to balance the centrifuge during centrifugation. Balancing tube of the same capacity can be adopted for balancing the centrifugal cryovial;

6.1.5.5 Wait until the centrifugation complete before opening the centrifuge.

6.1.6 Quality control of blood spots cards

Add sufficient blood onto the filter paper to fill the marked ring. Do not drip blood within the same ring several times; avoid touching or smearing the blood spot (figure below shows a qualified blood spot).
6.1.7 Unqualified blood spots

6.1.7.1 Sample size is too small: filter paper is removed before absorption complete; blood is dripped by capillary pipette; filter paper is touched before blood collection (especially with soap remnants on hand);

6.1.7.2 Samples are scratch: blood is dripped by capillary pipette;

6.1.7.3 Samples were collected without complete dryness;

6.1.7.4 Sample size is too large: unqualified dripping equipment; sample is dripped on both sides of the filter paper;
6.1.7.5 Faded or contaminated samples: sample collection area is extruded; sample collection area is touched by hand; blood spots are directly heated;

6.1.7.6 Appearance of serum ring: alcohol is not fully wiped off before sample collection; collection area is exposed to alcohol or hand sanitizer; intensive squeezing around blood collection area; samples are dried inappropriately; blood is stripped by capillary pipette;

6.1.7.7 Appearance of coagulation and stratification: blood is dripped within the same ring several times or on both sides of the filter paper;

6.1.7.8 No sample added:

6.2 On-site Blood Test

6.2.1 Whole blood separated by EDTA-3K is used for routine blood test (a three-or five-part automated hematology analyzer), which includes at least testing of Hb, WBC, RBC, PLT, and glycated hemoglobin HbA1c (by chromatography).
6.2.2 3# serum separated by vacuum tube with separation gel are used for ALT, TP, ALB, TC, TG, Glu and other biochemical items testing (using automated biochemistry analyzer).

6.3 Shipping and Preservation of Blood

6.3.1 On-site transport and preservation of specimens

Store the collected blood samples into freezer (2-8 °C) immediately, with a ratio of sample to dry ice be 1:1. Samples should be sent to local laboratory for further testing and processing within three hour; refrigerators are recommended for sample preservation; Samples Transfer Record Sheet (Appendix 1) and Samples Preservation Temperature Record Sheet (Appendix 4) should be attached to each batch of samples.

Freezer
Refrigerator

6.3.2 Preservation of samples in local laboratory

Carefully complete the sample temperature record sheet (Appendix 4) if samples are temporarily stored in local laboratory. All samples should be placed in condition of 2-8 °C. Samples cannot be stored in either -20 °C or -70 °C.

6.3.3 Transportation and preservation of samples in Provincial Laboratory

Upon completion of sample collection in local laboratory, samples should be transferred to provincial laboratory for preservation within TWO days. Freezers or refrigerators are required in transportation, and cold-chain transportation is strongly recommended. Samples should be received by technicians in provincial laboratory, and sample transfer record sheet (Appendix 1) and sample temperature record sheet (Appendix 4) need to be completed afterwards. Whole blood samples need to be preserved in 2-8 °C condition, while all other samples in -70 °C condition (Note: no sample can be preserved in -20 °C condition).

6.3.4 Transportation and preservation of samples in Beijing Central Laboratory
6.3.4.1 Blood samples sent to provincial laboratories need to be monitored by specific staff, who are responsible for timely delivery of samples to central laboratory of China-Japan Friendship Hospital.

6.3.4.2 **Option One**: Refrigerators or sample preservation boxes are ideal for sample delivery. Provinces adopting this method should have technicians monitor storage temperature during the period of transportation.

6.3.4.3 **Option Two**: For distant provinces, cooperation with cold-chain logistics companies experienced in specimens transportation is expected. **Temperature control during the delivery process is critical, and LCDs should be applied if necessary.**

*Note: consultation with Chins-Japan friendship hospital should be made before sample delivery.*

6.3.4.4 Samples sent to the central laboratory should be preserved by designated technicians, who are responsible for regular inspection and record keeping. Complete kept by the designated laboratory hand, regular inspection and take notes. Complete *Sample Transfer Record Sheet* (Appendix 1) and *Samples Preservation Temperature Record Sheet* (Appendix 4).

6.3.4.5 China-Japan Friendship Hospital will use the Frozen Samples Management System to manage provincial samples systematically.

(1) Method of sample storage in Frozen Samples Management System

Frozen frame in ultra-low temperature freezer

Cryovial box in frozen frame

(2) Frozen Samples Management System coding rules

Location of specimen will be expressed in the form of AA-BB-CC-DD. Specifically, refrigerators are numbered AA; rows and columns of frozen frame inside freezer are numbered BB; rows and columns of cryovial boxes inside the frozen frame are numbered CC; location inside cryovial boxes are numbered DD.
(3) Frozen Samples Management System software

Specimen management software allows storage conditions of all main refrigerators shows on screen, and can easily register database, search, statistics, and access to laboratory operations. Quick accesses of importing and exporting data are also available with bar code scanning system.

(4) Temperature monitoring program

Temperature data of several refrigerators can be captured and monitored in computer through the interface of control board's RS-485. Wired or wireless connection enables Long-term record of data, and sound and light alerts will be conducted in unusual circumstance.
省中心实验室保存 → 运输包装

方案一：专车运输

方案二：物流公司

航空快运 → 铁路快运 → 公路冷链

北京中心实验室样本冻存管里系统保存
6.4 Quality Control in Shipping and Preservation

6.4.1 On-site blood samples should be stored in freezers or in 2-8 ℃ in a timely manner;

6.4.2 Blood centrifugation should be completed within 3 hours after collection process.

6.4.3 All blood samples should be processed and preserved in cryovials according to the Manual of blood processing. Cryovials holding whole blood samples (blue-stoppered) should be preserved at room temperature and in 2-8 ℃; cryovials holding the buffy coat (white-stoppered) should be preserved in -70 ℃, and cannot be preserved in -20 ℃; other samples should be kept frozen at -70 ℃;

6.4.4 All frozen samples after centrifugation should be transferred to destination as soon as possible to avoid melting;

6.4.5 Sample processing and handling should stick strictly to the manual;

6.4.6 Supervisors should conduct random sample inspection, with a good pass rate reaching 98%. The inspection includes barcodes labeling, sample packing, and information presented in Samples Preservation Information Form (Appendix 5);

6.4.7 Clearly record all information in Samples Transfer Record Sheet (Appendix 1) during the samples transfer process;

6.4.8 Strictly control the preservation condition, and clearly record all information in Samples Preservation Temperature Record Sheet (Appendix 4).
7 ITEMS AND METHODS IN BLOOD TEST

7.1 Items and Methods in On-site Blood Test

On-site blood testing of this survey includes routine blood test (using 3 or 5 classification automated hematology analyzer), including at least Hb, WBC, RBC, PLT and serum ALT, TP, ALB, TC, TG, Glu and other biochemical items (using automated biochemistry analyzer), and glycated hemoglobin HbA1c (by chromatography).

All on-site testing items except Glu, Hb, and HbA1c, obtained for further analysis and records in Beijing central laboratory, are testing results reported to volunteers that need no further processing.

7.1.1 Method of routine blood test: 3 or 5 classification automated hematology analyzer
(The following procedures regard to Sysmex XE-2100D automated hematology analyzer. For method of specific machine, follow its SOP)

7.1.1.1 Measurement Principle:

XE-2100D performs hematology analyses according to the RF/DC detection method, Hydro Dynamic Focusing (DC Detection), flow cytometry method (using the semiconductor laser) and SLS-hemoglobin method. The XE-2100D can analyze and output the results for 32 parameters of blood samples. It utilizes technology of fluorescence flow cytometry to quantitate the standard five part differential, immature granulocytes (metamyelocytes, myelocytes and promyelocytes), nucleated red blood cells (NRBC), reticulocyte count, immature reticulocyte fraction and “optical” fluorescent platelet count. The combination of side scatter (inner complexity of the cell), forward scatter (volume) and fluorescence intensity of nucleated cells gives a concise but precise image of each cell detected in the peripheral blood. A well-defined physical description of the different leucocyte populations (clusters) is obtained. Abnormal and immature cells, with their larger nuclear volume show much higher fluorescence intensity than normal cells, and are easily distinguishable in the DIFF scatter gram.

7.1.1.2 Samples examination:

(1) Press the "MANUAL" button, and enter the sample ID number using the number keys;

(2) Select items to be examined from the list (Discrete);

(3) Place the sample on examines board, then press he green “START” button;
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(4) Remove the specimen when hearing short beep and observing the "READY" sign goes dark;

(5) When all tube racks are moved into left of the auto-sampler, the ready sign will light up, showing that the machine is ready for tests performing;

(6) Testing results can be obtained directly from the computer at any time during the automatic analysis process.

(7) Automatic transmission can be replaced by manual transmission if problem occurs.

7.1.1.3 Quality Control

(1) Choose at least two levels from high, medium, and low whole blood quality control materials provided by the SYSMEX Company;

(2) Quality control substance should be obtained from refrigerator and placed in stationary condition under room temperature for 15 minutes before the testing process. Twist the tube back and forth 10 times with hands, turn the tube upside down, and twist another 10 times. Repeat this process eight times, and then gently shake the tube for fully mix (one minute). Then conduct substance testing following regular procedure;

(3) Testing cycle: test each batch of samples once;

(4) Preservation temperature: 2-8 °C in refrigerator, avoid freezing;

(5) Allowable range: 3SD or manufacturers within the scope defined.

7.1.1.4 Requirements of specimen

(1) Specimen type: EDTA-3K whole blood;

(2) Container: EDTA-3K anticoagulant tube;

(3) Preservation: At room temperature, WBC, RBC, PLT can stay for 24hours, white blood cell can stay for 6-8hours, and Hb can stay for several days. While storing in 4 °C extends general preservation period of blood, platelets cannot be placed in such low temperature, for low temperature will affect testing result of PLT and MPV. If test cannot be conducted in a timely manner, blood should be kept at room temperature.

7.1.1.5 Testing results report (example is taken with three classification routine blood test. Items with * must be included)
### 7.1.2 On-site Glucose Test: AUTOMATED BIOCHEMISTRY ANALYZER

(The following procedures regard to serum glucose testing. For testing method of specific items, follow its SOP)

#### 7.1.2.1 Theory of testing (glucose oxidase)

Enzymatic method yields maximum specificity for glucose estimation. Glucose can be measured by its reaction with glucose oxidase, in which gluconic acid and hydrogen peroxide are formed. Hydrogen peroxide than reacts with oxygen acceptor phenylamine-phenazone and HSDA (Sodium N-(2hydroxy-3-sulfopropyl)-3.5-dimethoxyaniline) in a reaction catalyzed by peroxidase to form a color pigment. Absorbance is increased at 570nm, and glucose level can be obtained by measuring the absorbance changes.

#### 7.1.2.2 Sample test

(1) Start water source and water container, then conduct starting inspection (absorbance inspection, suction gauges check, reagents inspection, clean-up data);

(2) Conduct quality control;

(3) Sample test: Press [START] → on [START SAMPLE NO] → [SER / PI] enter the initial sample
series ID number → [ENTER] → [START] testing start, and [HOST SETTING] should be switched to [YES];

(4) Shut down and conduct daily maintenance.

7.1.2.3 Quality Control Substance

(1) Drip 5mL pure water (water quality > 15MΩ) with the Ostwald Folin Pipette (calibration only) onto the freezing dried quality control substance to form solution. Note that water shall not be blown back into the tube when using Ostwald Folin Pipette and the pipette should stay against the wall for 15 seconds;

(2) Tightly cover the tube and gently invert the tube for 5 times. Then allow the tube to be placed in station for 30 minutes. Gently mix the substance prior to use;

(3) Store dissolved quality control substance in 4-8 ℃ environment (can be kept for up to three days). For separate packaging, sealed with a double sealing membrane, then store in refrigerator in -20 ℃ (can be kept for up to one month);

(4) Testing cycle: test once with each batch of samples;

(5) method of quality control operation: take out the quality control substance and place I in room temperature for complete melting (takes about 20 minutes), then gently shake the tube five times. Perform calibration: Plot Levey-Jennings diagram according to target value and standard deviation; multi-rule quality control is frequently adopted.

7.1.2.4 Requirements of specimen

(1) Specimen type: tube with separation gel (Red top with yellow ring);

(2) Acquisition criteria: volunteers should live a life with normal eating habit, avoid fierce sporting or high mental pressure prior to the empty stomach blood collection process (avoid eating for at least eight hours, but no more than 16 hours).

(3) Storage conditions: regular tube contains no serum can be kept for 2 hours in room temperature; frozen serum for 24 hours of preservation.

(4) Note: regular tubes should be kept in freezer immediately after separating the serum; blood glucose will reduce 5% -7% / h if placed at room temperature.

7.1.2.5 Recommended method and on-site test results report

<table>
<thead>
<tr>
<th>Items</th>
<th>Chinese name</th>
<th>Reference range</th>
<th>unit</th>
<th>Recommended methods</th>
</tr>
</thead>
</table>

50
<table>
<thead>
<tr>
<th>Glu</th>
<th>葡萄糖</th>
<th>3.61-6.11</th>
<th>mol/L</th>
<th>Glucose Oxidase</th>
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<td>ALT</td>
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<td>Biuret</td>
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<td>Oxidase</td>
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<tr>
<td>TG</td>
<td>甘油三酯</td>
<td>&lt;1.70</td>
<td>mmol/L</td>
<td>Enzymatic process (dissociation)</td>
</tr>
</tbody>
</table>

7.1.3 Test of glycosylated hemoglobin (HbA1c)
Note: Test of glycosylated hemoglobin should be conducted in the central provincial laboratory, with one each province.

7.1.3.1 Measurement principle (high-performance liquid chromatography)

High-performance liquid chromatography (or high-pressure liquid chromatography, HPLC) is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture. HPLC typically utilizes different types of stationary phases, a pump that moves the mobile phase(s) and analyze through the column, and a detector that provides a characteristic retention time for the analyze. The detector may also provide other characteristic information. Analyze retention time varies depending on the strength of its interactions with the stationary phase, the ratio/composition of solvent(s) used, and the flow rate of the mobile phase. With HPLC, a pump (rather than gravity) provides the higher pressure required to propel the mobile phase and analyze through the densely packed column. The increased density arises from smaller particle sizes. This allows for a better separation on columns of shorter length when compared to ordinary column chromatography. Absorbance is measured at 415 nm.

7.1.3.2 Sample test

(1) Place the specimen on testing board in numerical order;

(2) Press MENU button, then press PARAMER button and select SAMPLE No, entering corresponding number (sample ID) using number keys; press ENTER button, then press START;

(3) Note that specimens must mix completely;

(4) As the process complete, STAT sign start to dim, and samples can be removed.

7.1.3.3 Quality Control Substance

(1) Drip 500 µl pure water (water quality> 15MΩ) with the Ostwald Folin Pipette (calibration only) onto the freezing dried quality control substance (5 µl) to form solution. Well mixed solution can be
used immediately. Store dissolved quality control substance in 4-8 °C environment (can be kept for up to seven days).

(2) Testing cycle: test once with each batch of samples

(3) Method of preservation: quality control substance that have not been used can be stored at 2-8 °C environment; re-dissolved quality control substance can be kept at temperature of 4-8 °C for seven days;

(4) Method of quality control operation: take out the quality control substance and place them in room temperature for complete melting (takes about 10 minutes), then gently shake the tube six times.

7.1.3.4 Requirements of specimen

(1) Specimen container: EDTA anticoagulant vacuum tube.

(2) Acquisition criteria: volunteers should live a life with normal eating habit; avoid fierce sporting or high mental pressure prior to sample collection.

(3) Storage conditions: one day in room temperature; seven days in freezing condition.

7.1.3.5 Results Report

7.1.3.6 Reference range

Reference range of Glycated hemoglobin is 5% to 8%.
7.2 Items and Methods in Beijing Central Laboratory
(Methods in accordance with SOP for Laboratory)

7.2.1 Test items and methods

<table>
<thead>
<tr>
<th>Items</th>
<th>Chinese name</th>
<th>Reference range</th>
<th>Unit</th>
<th>Recommended methods</th>
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<td>Glucose Oxidase</td>
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<td>&lt;5.20</td>
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<tr>
<td>LDL-C</td>
<td>低密度脂蛋白胆固醇</td>
<td>&lt;3.12</td>
<td>mmol/L</td>
<td>Enzymatic process (direct)</td>
</tr>
<tr>
<td>TG</td>
<td>甘油三酯</td>
<td>&lt;1.70</td>
<td>mmol/L</td>
<td>Enzymatic process (dissociation)</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>超敏 C 反应蛋白</td>
<td>0-3</td>
<td>mg/L</td>
<td>Latex agglutination</td>
</tr>
<tr>
<td>CRE</td>
<td>肌酐</td>
<td>35-106</td>
<td>μmol/L</td>
<td>Picric acid</td>
</tr>
<tr>
<td>ALB</td>
<td>白蛋白</td>
<td>35-55</td>
<td>g/L</td>
<td>Bromocresol green (BCG)</td>
</tr>
<tr>
<td>Apo-A1</td>
<td>载脂蛋白 A1</td>
<td>1.10-1.60</td>
<td>g/L</td>
<td>Immunoturbidimetry</td>
</tr>
<tr>
<td>Apo-B</td>
<td>载脂蛋白 B</td>
<td>0.70-1.20</td>
<td>g/L</td>
<td>Immunoturbidimetry</td>
</tr>
<tr>
<td>Lp（a）</td>
<td>脂蛋白（a）</td>
<td>&lt;0.3</td>
<td>g/L</td>
<td>Immunoturbidimetry</td>
</tr>
<tr>
<td>Urea</td>
<td>尿素</td>
<td>2.78-7.85</td>
<td>mmol/L</td>
<td>Urease test</td>
</tr>
<tr>
<td>UA</td>
<td>尿酸</td>
<td>150-420</td>
<td>μmol/L</td>
<td>Uric Acid-POD</td>
</tr>
<tr>
<td>Mg</td>
<td>镁</td>
<td>0.78-1.27</td>
<td>mmol/L</td>
<td>Xyline blue</td>
</tr>
<tr>
<td>TP</td>
<td>总蛋白</td>
<td>60-80</td>
<td>g/L</td>
<td>Biuret</td>
</tr>
<tr>
<td>TRF</td>
<td>转铁蛋白</td>
<td>187-312</td>
<td>mg/dL</td>
<td>Immunoturbidimetry</td>
</tr>
<tr>
<td>TRF-R</td>
<td>转铁蛋白受体</td>
<td></td>
<td>mmol/L</td>
<td>ELISA</td>
</tr>
<tr>
<td>Fet</td>
<td>铁蛋白</td>
<td>11-336</td>
<td>ng/mL</td>
<td>Immunoturbidimetry</td>
</tr>
<tr>
<td>INS</td>
<td>胰岛素</td>
<td></td>
<td>mmol/L</td>
<td>Immunoturbidimetry</td>
</tr>
</tbody>
</table>

7.2.2 Operation procedures of Hitachi 7600 in Beijing central laboratory

7.2.2.1 Preparation

Start water supply and container; set automatic maintenance after machine starts.

7.2.2.2 Starting Check

(1) Optics examination [UITLITI]: [MAINTENANCE] → [PHOTOMETER CHECK] → [SELECT] → [EXECUTE];
(2) Print the results analysis. If an output level of 340nm > 19,000, light source need to be updated. See Mid-year Maintenance Manual for detailed procedure. Contact maintenance service if the difference in wave length between dominant wave and secondary wave has an output level exceeds 10;

(3) Suction gauges check (air bubbles and leakage). If bubbles exist, do the following: [UİTLİTİ]: [MAİNTENANCE] → [AIR PURGE] → [SELECT] → [EXECUTE];

(4) Reagents check: Pressing [REGENT] to obtain reagent information: measure number of reagents, detected remaining quantity of each type of reagents. Add additional materials to insufficient reagents.

(5) Data clean-up: [WORKPLACE] → [DATE REVIEW] → [BATCH DELETE] → [ALL] → [OK].

7.2.2.3 Quality control

(1) Place quality control materials on the white shelf C, light-colored lower values on C1, dark-colored higher values on C2, then lay down the handle.

(2) Press [QC] → select all items appeared in [TEST] → [POSELECT] → [SAVE] → press [START] → large [START] machine starts to manipulate quality control process. Examine the output of quality control: if the result does not lie within the range of 3SD, reschedule the calibration.

7.2.2.4 Calibration

(1) [CALIBRATION] → [START UP] → conduct blank test [BLANK] → conduct calibration test [2POINT] → [OK];

(2) Place NS on the standard rack S001 (black) position 1, CSF multi-standards on the same rack at position 2 (ApoA.B, HDL, LDL, Lpa are each placed at its specific position following standard rules) position the standard rack and lie down the propeller;

(3) Press [SELECT START UP CALIBRATION] → [CHANGE] → [START UP CALIBRATION] → [OK] switch to [YES] → press [START] to start calibration process. Conduct quality control test of different levels after calibration complete, and start the machine within controlled range.

7.2.2.5 Importing: [WORKPLACE] → [NORMAL TS] → [SAMPLE NO.] → Enter specimen ID number → Enter testing items → [SAVE].

7.2.2.6 Specimen preparation: The Deal samples into the sample holder according to the order of, the right seat. Note: The samples are to be placed to avoid touching pins, will advance to put down.
7.2.2.7 The test samples: according to [START] → in [START SAMPLE NO] → [SER / PI] enter the starting sample number → [ENTER] → [START] beginning of instruments, and should [HOST SETTING] into [YES].

7.2.2.8 Data transfer: In general, pressing [HOST SETTING] and switch to [YES] at the beginning of the analysis can have the measurement results automatically delivered; if the data transfer failure occurs, re-transfer the data following these steps: pressing [DATA REVIEW] → select all data to be transferred → [SEND HOST] → [YES].

7.2.2.9 Maintenance:

(1) Operators recycle all samples from the rack in order, and put samples back into refrigerator for preservation after verification.

(2) Pour away all liquid waste, add reagents, and clean the reaction flask with proper lotion.

7.2.2.10 shutting down:

(1) Press the orange power button on the lower left corner for several minutes to turn off the machine.

(2) Clean reagent needles, sample needles, and stirring rods.

(3) Disinfect equipment surfaces, remove specimens off the sample rack in order, then record date and store all specimens in 2-8°C environment.

(4) Record and sign for equipment usage and status.

7.2.3 Quality Control in Blood Samples Test

7.2.3.1 Drip 5mL pure water (water quality > 15MΩ) with the Ostwald Folin Pipette (calibration only) onto the freezing dried quality control substance to form solution. Note that water shall not be blown back into the tube when using Ostwald Folin Pipette and the pipette should stay against the wall for 15 seconds;

7.2.3.2 Tightly cover the tube and gently invert the tube for 5 times. Then allow the tube to be placed in station for 30 minutes. Gently mix the substance prior to use;

7.2.3.3 Store dissolved quality control substance in 4-8 °C environments (can be kept for up to three days). For separate packaging, sealed with a double sealing membrane, then store in refrigerator in -20 °C (can be kept for up to one month);

7.2.3.4 Testing cycle: test once with each batch of samples;
7.2.3.5 Method of quality control operation: take out the quality control substance and place I in room temperature for complete melting (takes about 20 minutes), then gently shake the tube five times. Perform calibration: Plot Levey-Jennings diagram according to target value and standard deviation; Westgard multi-rule quality control is frequently adopted.

7.2.4 Requirements of specimen

7.2.4.1 Specimen Type: tube with separation gel (Red top with yellow ring);

7.2.4.2 Acquisition criteria: volunteers should live a life with normal eating habit, avoid fierce sporting or high mental pressure prior to the empty stomach blood collection process (avoid eating for at least eight hours, but no more than 16 hours).

7.3 Quality control in blood sample test

Quality control in China Health and Nutrition Examination Survey Project (2009 CHNS) is completed in three parts:

Part I: Training assessment and rating of technicians and staff prior to the survey starts;
Part II: quality control in on-site laboratory: constant value quality control;
Part III: quality control in central laboratory: Research Center of total quality control laboratory - like blind investigation.

7.3.1 Training assessment and rating prior to the survey

Preselected quality control substance (unknown) will be issued towards each site from central laboratory. Supervisor of each site should conduct quality control test 10 times and fax the result to the central laboratory as soon as possible. After receiving data from all provinces, assessment will take place in central laboratory using Deviation Index (DI) method. (Take fixed percentage of target value as measuring standard, referred to as modified deviation index). Set deviation from target value be 5%, that is, DI = 1.

DI calculation formula:

\[
DI = \frac{|X - \bar{X}|}{X} \times 100\% = \frac{|X - \bar{X}|}{X} \times 20
\]

\(\bar{X}\) in the formula represents mean value of all data, with X as a standard blood glucose levels and 5% be the bias ratio (constant) (in the calculation of blood sugar, 5% equals 1DI). This formula obtains absolute value of DI, which indicates the following test results:

- DI ≤ 0.5 = Excellent
- 0.5 < DI ≤ 1.0 = Good
- 1.0 < DI ≤ 1.6 = Pass
- DI > 1.60 = Fail
Note: The technical evaluation is designed for technicians to know the accuracy of their test results, identify problems, and get improvement.

7.3.2 Quality control within the laboratory (internal quality control) – constant value quality control method

Constant value quality control can be used to monitor the precision and accuracy of test, as well as indicating the size and type of error in a timely manner. The survey requires the local laboratory to provide its Levey-Jennings chart for one month, and fill out the "routine quality control chart" (see Appendix 12) setting the value of quality control chart rules are as follows:

1. On the x-axis the date and time, or more usually the number of the control run, are plotted. A mark is made indicating how far off the actual result was from the mean (which is the expected value for the control). The distance from the mean is measured in standard deviations (SD). Lines run across the graph at the mean, as well as one, two (green line represents caution) and three (red line represents control limit) standard deviations either side of the mean.

2. Measure substance control at different levels horizontally and mark on the graph correspondingly before the test start. Take the average value and draw lines that link together all neighbor dots.

3. All dots should lie within the caution line. Dots lie beyond the caution line and within the control line are considered relevant dots with significant errors, and further examination are expected to be drawn. Dots lie beyond the control line are considered irrelevant outliers that need to be deleted from the samples.

4. Repeated measurement of the length of vertical connections indicates degree of precision, with longer measure result representing less accuracy. Average value points’ distance to the standard line indicates accuracy of the test, with shorter distance representing better accuracy. Precision and accuracy of the testing results can be judged upon length of vertical connections and scattering of average value points.

7.3.3 Total quality control in Central Laboratory --- Unknown constant value quality control

National central laboratory issues unknown quality control substance uniformly, which needs to be tested once or twice with each batch of samples, and the results are to be recorded in on-site laboratory information record sheet. The on-site laboratory information record sheet should be sent back to Beijing central laboratory after all tests are completed.
8 WORK SITE CLEAN-UPS AND SUMMARY

8.1 Documentation of Questionnaires and Forms

8.1.1 Staff in the investigation team should verify and collect all questionnaires, revising erroneous columns and cover missing items. Signatures of team leaders indicate completion of the verification process;

8.1.2 All questionnaires and forms involved in the survey should be completed and confirmed by team leaders. Signatures are required for documentation.

8.1.3 Check all paper documents and complete the on-site collection summary form (Appendix 6).

8.2 Samples Summary

8.2.1 Check the sample bar code

8.2.2 Check storage location of packing samples, bar code information, and then fill out the sample storage information sheet;

8.2.3 Check all samples and complete the on-site collection summary form (Appendix 6).

8.3 Supplies clean-ups

We use each a bag of supplies in the survey program. Consumable supplies before and after survey change bag as follows

<table>
<thead>
<tr>
<th>Before samples collection</th>
<th>After samples collection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Item</strong></td>
<td><strong>Quantity</strong></td>
</tr>
<tr>
<td>EDTA vacuum blood collection tubes</td>
<td>1</td>
</tr>
<tr>
<td>Vacuum blood collection tubes with separation gel</td>
<td>2</td>
</tr>
<tr>
<td>Yellow-stoppered cryovials</td>
<td>4</td>
</tr>
<tr>
<td>Blue-stoppered cryovial</td>
<td>1</td>
</tr>
<tr>
<td>Green-stoppered cryovial</td>
<td>1</td>
</tr>
<tr>
<td>White-stoppered cryovial</td>
<td>1</td>
</tr>
<tr>
<td>Red-stoppered cryovial</td>
<td>1</td>
</tr>
<tr>
<td>Blood spots card</td>
<td>1</td>
</tr>
<tr>
<td>Extra barcode labels (includes five on-site barcodes labels and two extra barcode labels)</td>
<td>1</td>
</tr>
<tr>
<td>Toenails collection envelop (contains toenails)</td>
<td>1</td>
</tr>
</tbody>
</table>
8.4 Medical Waste Cleanup and Bio-safety

Since the survey involves minimally invasive injury and associated medical waste generation, bio-safety management is especially important during blood sample collection. As a result, recycling equipment such as medical waste collection bags and bins should be prepared in advance.

8.5 Feedback

Feedbacks of the on-site experiments are expected to be delivered directly in the same day. Privacy of volunteers is highly protected during the whole process, and no disclosure of the testing results is allowed.

Collection of biological samples in CHNS, including sample collection, processing, preservation, transportation and determination and many other processes, should be conducted strictly according to the operation requirements stated in this manual. High quality of the completion of all tasks is expected during the whole process.
Appendix

Appendix 1: Samples Transfer Record Sheet
Appendix 2: On-site Laboratory Information Form
Appendix 3: Basic Information Verification Form
Appendix 4: Samples Preservation Temperature Record Sheet
Appendix 5: Samples Preservation Information Form
Appendix 6: On-site Collection Summary Sheet
Appendix 7: Dietary Directory
Appendix 8: Venipuncture Tips
Appendix 9: Informed Consent
Appendix 10: Blood Sample Collection Questionnaire
Appendix 11: Toenails Collection Questionnaires
Appendix 12: Routine Internal QC Plan
Appendix 13: Coding Chart

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