**Supplemental Table 1.** Preanalytical conditions required to obtain a valid sample for laboratory analysis1

|  |  |  |  |
| --- | --- | --- | --- |
| **Indicator** | **Sample collection** | **Sample processing** | **Sample storage** |
| SF and sTfR | Venous and capillary samples comparable but higher variability with capillary samples (1) | Prepare serum within 1–3 d of blood collection (2); avoid exposure to elevated temperature (3;4) | Stable for ≤14 d at 4°C (4); stable for at least 1 year at ≤-20°C (5)Stable for at least 3 freeze/thawing cycles (4) |
| Iron | Venous samples are typically used because capillary samples do not provide sufficient sample volume for clinical analyzer | Prepare serum same day as collected (6); avoid exposure to elevated temperature (3) | Stable for ≤7 d at 4°C (7); stable for at least 1 year at ≤-20°C (5)Stable for at least 3 freeze/thawing cycles (in-house data) |
| EP | Venous and capillary samples comparable; avoid partially clotted samples (8) | Protect sample from UV light (8); avoid contamination with fluorescent interferences (9;10); fresh vs. frozen blood depends on method | Stable for ≤10 d at 4°C if protected from UV light (8); stable for months at ≤-20°C (8)Stable for at least 3 freeze/thawing cycles (in-house data) |
| Hb | Venous and capillary samples comparable but higher variability with capillary samples (11;12) | No processing needed; intact whole blood is analyzed | Stable for 1–2 days at 4°C (11) |

1 EP, erythrocyte protoporphyrin; Hb, hemoglobin; SF, serum ferritin; sTfR, soluble transferrin receptor.

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**Supplemental Table 2.** Less common analytical methods to measure biochemical iron status indicators1

|  |  |  |  |
| --- | --- | --- | --- |
| **Indicator** | **Assay** | **Advantages** | **Disadvantages** |
| SF and sTfR | ELISA (commercial kit or in-house developed) | Requires only small sample volume (≤100 µL); relatively inexpensive instrumentation (microplate reader and plate washer) | Manual procedure requires several pipetting steps and adherence to strict timing; limited sample throughput; moderate precision (duplicates needed); moderately expensive reagent costs for commercial kits; in-house assays need to be carefully validated; assays rely on continued availability of suitable antibodies |
| Iron and TIBC2 | Manual colorimetric assay using ferrozine as chromogen | Relatively inexpensive instrumentation (photometer), reagents and supplies | Manual multi-step assay; high sample volume (≥500 µL serum); moderate precision; need for rigorous elimination of iron contamination |
| Iron | Atomic absorption spectrophotometry and more recently inductively coupled plasma mass spectrometry | “Reference-type” assay; relatively simple processing; quick analysis time; good precision | Relatively expensive instrumentation requires regular maintenance and periodic technical services; not commonly available in clinical laboratories |
| EP3 | Fluorometry after extraction | Frozen EDTA blood can be used; washed erythrocytes can be used to remove fluorescent interferences; small sample volume (≤20 µL); relatively inexpensive instrumentation | Manual multi-step assay requires chemicals that pose safety hazards; moderate precision (duplicates needed); prone to external fluorescent contamination; work under subdued light conditions; need hematocrit to correct for packed red cells |
| ZPP | Hematofluorometer | Simple and inexpensive instrumentation; can be set up in the field, but not portable; requires only one drop of blood | Requires freshly collected blood; moderate precision; fluorescent interferences possible; instrument performance not robust |

1 ELISA, enzyme linked immunosorbent assay; EP, erythrocyte protoporphyrin, measured as free EP; SF, serum ferritin; sTfR, soluble transferrin receptor; TIBC, total iron-binding capacity; UIBC, unsaturated iron-binding capacity; ZPP, zinc protoporphyrin.

2 Used in NHANES 1971–2000.

3 Used in NHANES 1976–2006.

**Supplemental Table 3.** Iron status indicators measured in NHANES surveys, 1971–20161

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Indicator** | **Matrix** | **I** **1971**–**1975** | **II** **1976**–**1980** | **Hispanic****1982**–**1984** | **III** **1988**–**1994** | **1999**–**2002** | **2003**–**2006** | **2007**–**2010** | **2015**–**2016** |
| SF | Serum |  | (✓)2 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| TSAT |  | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |  |  |
|  Iron | Serum | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |  |  |
|  TIBC | Serum | ✓ | ✓ | ✓ | ✓ | ✓ |  |  |  |
|  UIBC | Serum |  |  |  |  |  | ✓ |  |  |
| EP | Whole blood |  | ✓ | ✓ | ✓ | ✓ | ✓ |  |  |
| sTfR | Serum |  |  |  | (✓)3 | (✓)4 | ✓ | ✓ | ✓ |
| Hb and Hct | Whole blood | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Other red cell indices5 | Whole blood |  | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| **Population** |  |  |  |  |  |  |  |  |  |
| Biochemical indicators |  | All ages, M+F | All ages, M+F |  | All ages, M+F | All ages, M+F | 1–5 y, WRA | 1–5 y, WRA | 1–5 y, WRA |
| Hematologic indicators |  | All ages, M+F | All ages, M+F |  | All ages, M+F | All ages, M+F | All ages, M+F | All ages, M+F | All ages, M+F |

1 No biochemical iron status indicators were measured during 2011–2014; EP, erythrocyte protoporphyrin (measured as free EP after extraction); Hb, hemoglobin; Hct, hematocrit; F, females; M, males; SF, serum ferritin; sTfR, soluble transferrin receptor; TIBC, total iron-binding capacity; UIBC, unsaturated iron-binding capacity; WRA, women of reproductive age ages 12–49 y.

2 SF measured on a representative subsample of 5157 individuals ages 3–74 y.

3 sTfR measured on a convenience sample of 2186 individuals ages ≥3 y from 13 survey locations.

4 sTfR measured in pregnant women as part of a surplus serum project.

5 Other red cell indices measured were MCV, MCHC and RDW (starting with NHANES III 1988–1994).

**Supplemental Table 4.** Methods used to measure iron status indicators in NHANES surveys, 1971–20161

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Indicator** | **Matrix** | **I****1971**–**1975** | **II****1976**–**1980** | **Hispanic****1982**–**1984** | **III****1988**–**1994** | **1999**–**2002** | **2003**–**2006** | **2007**–**2010** | **2015**–**2016** |
| SF | Serum |  | IRMA2 | BioRad IRMA | BioRad IRMA | BioRad IRMA | Hitachi 912 IT3 | Roche E-170 IT | Roche E-170 IT |
| TSAT |  | Calculated Iron/TIBC | Calculated Iron/TIBC | Calculated Iron/TIBC | Calculated Iron/TIBC | Calculated Iron/TIBC4 | Calculated Iron/(Iron+UIBC) |  |  |
|  Iron | Serum | Manual colorimetric | Manual colorimetric | Manual colorimetric | Manual colorimetric | Manual colorimetric4 | Beckman LX20 colorimetric |  |  |
|  TIBC | Serum | Manual colorimetric | Manual colorimetric | Manual colorimetric | Manual colorimetric | Manual colorimetric4 |  |  |  |
|  UIBC | Serum |  |  |  |  |  | Beckman LX20 colorimetric |  |  |
| EP | Whole blood |  | Fluorescence extraction5 | Fluorescence extraction5 | Fluorescence extraction5 | Fluorescence extraction5,6 | Fluorescence extraction6 |  |  |
| sTfR | Serum |  |  |  | ELISA7 | Hitachi 912 IT | Hitachi 912 IT | Roche Mod P IT | Roche Mod P IT |
| Hb | WB | Coulter FN | Coulter FN | Coulter ZBI | Coulter S Plus Jr | Coulter MAXM | Coulter MAXM | Coulter MAXM | Coulter MAXM |
| Hct | WB | Centrifuge | Centrifuge | Centrifuge | Coulter S Plus Jr | Coulter MAXM | Coulter MAXM | Coulter MAXM | Coulter MAXM |
| Other red cell indices | WB |  | Calculated | Calculated | Coulter S Plus Jr | Coulter MAXM | Coulter MAXM | Coulter MAXM | Coulter MAXM |

1 Laboratory analyses for biochemical indicators were conducted by the CDC Nutritional Biomarkers Laboratory unless otherwise indicated; laboratory analyses for hematologic indicators were conducted at the Mobile Examination Center; no biochemical iron status indicators were measured during 2011–2014; ELISA, enzyme linked immunosorbent assay; EP, erythrocyte protoporphyrin (measured as free EP after extraction); IRMA, immunoradiometric assay; IT, immunoturbidimetry; Hb, hemoglobin; Hct, hematocrit; SF, serum ferritin; sTfR, soluble transferrin receptor; TIBC, total iron-binding capacity; UIBC, unsaturated iron-binding capacity.

2 University of Kansas Medical Center (laboratory of James D. Cook, M.D.).

3 BioRad IRMA was used in 2003; data were adjusted to Hitachi 912 IT assay prior to release.

4 Collaborative Laboratory Services (laboratory of David Witte, M.D., Ph.D.) analyzed samples for NHANES 2002 with the same methods as for NHANES 2003–2006.

5 CDC modification of Sassa & Granick method was used during 1976–2001 (Sassa S, Granick JL, Granick S, Kappas A, Levere RD. Microanalysis of erythrocyte protoporphyrin levels by spectrophotometry in the detection of chronic lead intoxication in the subclinical range. Biochem Med 1973;8:135–48).

6 Wadsworth Center, NY State Department (laboratory of Patrick Parsons, Ph.D.) analyzed samples for NHANES 2002–2006 with the Sassa & Granick method.

7 University of Kansas Medical Center (laboratory of James D. Cook, M.D.).