

Assessing the **IRON STATUS** of populations

Report of a Joint
World Health Organization/
Centers for Disease Control and Prevention
Technical Consultation on the Assessment
of Iron Status at the Population Level

Geneva, Switzerland
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1. Consultation

1.1 Rationale for the Consultation

Anaemia is one of the most common and intractable nutritional problems in the world today. The World Health Organization (WHO) estimates that some two billion people are anaemic defined as haemoglobin concentrations that are below recommended thresholds. The main causes of anaemia are: dietary iron deficiency; infectious diseases such as malaria, hookworm infections and schistosomiasis; deficiencies of other key micronutrients including folate, vitamin B₁₂ and vitamin A; or inherited conditions that affect red blood cells (RBCs), such as thalassaemia.

Iron deficiency with or without anaemia has important consequences for human health and child development: anaemic women and their infants are at greater risk of dying during the perinatal period; children's mental and physical development is delayed or impaired by iron deficiency; and the physical work capacity and productivity of manual workers may be reduced. There have been many efforts to fight iron deficiency and anaemia over the past two decades but, despite these efforts, the conditions are still common.

One of the reasons for the apparent failure to reduce the prevalence of anaemia is that many programmes and their interventions have been designed with the assumption that the only cause of anaemia is iron deficiency. This has meant that, when trying to control anaemia, the role of other causes has been underestimated, and that iron deficiency without anaemia has not been addressed as a major and common health problem.

In the absence of international agreement on how to assess the iron status of populations, the prevalence of iron deficiency has often been derived from the prevalence of anaemia using measurements of blood haemoglobin concentration. However not all anaemic

mic people are iron deficient and iron deficiency may occur without anaemia. This means that the prevalence of anaemia and iron deficiency varies in different populations and no consistent relationship between the two can be applied throughout the world. When anaemia is considered from the point of view of programmes to improve nutrition and health, an estimate of the prevalence derived from the haemoglobin concentration alone does not allow the contribution of iron deficiency to anaemia to be estimated, and ignores the role of other causes of anaemia.

To plan effective interventions to combat both iron deficiency and anaemia there is an urgent need to have better information on the iron status of populations. This will enable the right interventions to be chosen in the first place and then, once programmes are in place, to have the right indicators to monitor their impact.

These were all reasons for holding the Joint WHO/Centers for Disease Control and Prevention (CDC) Technical Consultation on the Assessment of Iron Status at the Population Level. The Consultation took place in Geneva, Switzerland, from 6 to 8 April 2004.

1.2 Objectives of the Consultation

The objectives of the Consultation were:

- to review the indicators currently available to assess iron status;
- to select the best indicators to assess the iron status of populations;
- to select the best indicators to evaluate the impact of interventions to control iron deficiency in populations;
- to identify priorities for research related to assessing the iron status of populations.

2. Working definitions of key terms

For the sake of clarity and to achieve a consensus, several key terms were defined during the Consultation.

In clinical terms *anaemia* is an insufficient mass of RBCs circulating in the blood; in public health terms *anaemia* is defined as a haemoglobin concentration below the thresholds given by WHO, UNICEF, UNU (1). These thresholds are set at the 5th percentile of the haemoglobin concentration of a normal population of the same sex and age group. There is a separate threshold for pregnant women.

Although iron deficiency is probably the most common cause of anaemia, there are other causes as well, including: acute and chronic infections that cause inflammation; other micronutrient deficiencies, especially of folate, vitamin B₁₂ and vitamin A; and genetically inherited traits such as thalassaemia.

Iron deficiency is a state in which there is insufficient iron to maintain the normal physiological function of tissues such as the blood, brain, and muscles. Iron deficiency can exist in the absence of anaemia if it has not lasted long enough or if it has not been severe enough to cause the haemoglobin concentration to fall below the threshold for the specific sex and age group (1). Evidence from animals fed on iron-deficient diets indicates that iron deficiency becomes detectable at about the same time in the blood, brain, and tissue enzyme systems (2).

Storage iron is the pool of iron in the body that is not being used by tissues. Healthy children and adults (apart from infants aged 6–11 months and pregnant women) usually have some iron stores to act as a buffer against iron deficiency during periods when dietary iron may be temporarily insufficient. *Iron depletion* is the state in which storage iron is absent or nearly absent but the tissues that need iron are able to maintain normal physiological functions.

It is possible for a *functional iron deficiency* to develop even when iron stores are present if the normal physiological systems for transporting iron to target tissues are impaired. This occurs most commonly because of cytokines released during inflammation caused by infectious diseases, and appears to be mediated by hepcidin (3). Iron supplementation or fortification has no benefit in such circumstances. Deficiencies of other nutrients such as vitamin A may also cause a functional iron deficiency even when iron stores are adequate (4).

3. Selection of indicators

In January 2004 a WHO/CDC working group met to review the literature on indicators of iron status and to select the indicators considered to be the best for discussion by the Consultation. Appendix 1 shows the indicators considered, each of which was evaluated in terms of its theoretical advantage as an indicator of iron status and the practicality of its measurement. Appendix 2 shows the five indicators selected to assess iron status and an acute phase protein with the methods most commonly used to measure them. The rationale for selecting these five was as follows.

- Haemoglobin concentration is a measure of anaemia, a condition that has important outcomes for health and child development that are linked to international development goals.
- Zinc protoporphyrin reflects a shortage in the supply of iron in the last stages of making haemoglobin so that zinc is inserted into the protoporphyrin molecule in the place of iron. Zinc protoporphyrin can be detected in RBCs by fluorimetry and is a measure of the severity of iron deficiency.
- Mean cell volume indicates whether RBCs are smaller than usual (microcytic), which is a common sign of iron deficiency anaemia, or larger than normal (macrocytic), a common sign of megaloblastic anaemia resulting from a deficiency of vitamin B₁₂ or folate.
- Transferrin receptor in serum is derived mostly from developing RBCs and so reflects the intensity of erythropoiesis and the demand for iron; the concentration rises in iron deficiency anaemia and it is a marker of the severity of iron insufficiency only when iron stores have been exhausted, provided that there are no other causes of abnormal erythropoiesis. The concentration of transferrin receptor is also increased in haemolytic anaemia and

thalassaemia. Clinical studies indicate that the serum transferrin receptor is less affected by inflammation than serum ferritin (5).

- Serum ferritin is a measure of the amount of iron in body stores if there is no concurrent infection: when the concentration is $\geq 15 \mu\text{g/l}$ iron stores are present; higher concentrations reflect the size of the iron store; when the concentration is low ($< 12\text{--}15 \mu\text{g/l}$) then iron stores are depleted. When infection is present the concentration of ferritin may increase even if iron stores are low; this means that it can be difficult to interpret the concentration of ferritin in situations in which infectious diseases are common.

4. Literature reviews

The Consultation was provided with literature reviews on indicators of iron status, including RBC parameters, ferritin, free erythrocyte protoporphyrin, serum and plasma iron, total iron binding capacity, transferrin saturation and serum transferrin receptor as well as a review on the interpretation of indicators of iron status during an acute phase response. These reviews, which will be published separately, provide technical background to the measurement, biology, interpretation and diagnostic value of the indicators.

5. Analysis of data from iron intervention studies

In order to assess the potential of indicators to detect a change in iron status as a result of an intervention, the Consultation reviewed the results of an analysis of indicators of iron status and acute phase proteins that were measured during 10 double-blind, randomized controlled trials. The investigators provided iron either as supplements or as food fortified with iron for periods between 4 and 18 months to infants (1 study), preschool children (1 study), schoolchildren (2 studies), pregnant women (2 studies) and non-pregnant women (4 studies). The studies were done in Côte d'Ivoire (6), Jamaica (Simmons et al., unpublished data), Morocco (7), the Philippines (Beard and Haas, unpublished data), one study done in Sweden and Honduras (8), one study in the United Republic of Tanzania (9), two studies in the United States of America (Beard, unpublished data and 10), and two studies in Viet Nam (Thuy et al., unpublished data and 11). The original data sets from all trials were provided for this analysis by the investigators, who are acknowledged at the end of this report. Full details of the analysis will be submitted for publication in due course.

The haemoglobin and serum ferritin concentrations were measured in all ten trials, serum transferrin receptor in nine, zinc protoporphyrin in six, and mean cell volume by flow cytometry in four. For the nine studies that measured both serum ferritin and transferrin receptor, the body iron stores were estimated using the method and constants given by Cook, Flowers, Skikne (12). Because both serum ferritin and transferrin receptor concentrations have distributions skewed to the right, both raw values and values transformed to logarithms were used in the analysis.

5.1 Indicators for evaluating the impact of interventions to control iron deficiency

The data from the ten studies were selected because the experimental designs were considered to be adequate (duration of intervention, iron dosage and compound) to show an improvement in iron status. The data were used to assess how well each indicator predicted the changes in iron status. This change was estimated by calculating the mean difference between each indicator at the beginning and end of each study for the intervention group and then subtracting the mean difference calculated for the control group. As each indicator had different units, the net difference between the change in the intervention and control groups was divided by the standard deviation of the baseline measurement of the indicator calculated for both study groups in order to express it in standard deviation units (SDUs). For the purposes of analysis an arbitrary change of ≥ 0.2 SDUs was defined as indicating a successful response to the intervention. A power calculation indicated that this change could be detected with a sample size of 400 subjects per study group.

In addition, using the same studies, the change that occurred in the subjects with the lowest 10% of values was examined for indicators that were expected to rise, such as haemoglobin concentration, and for the highest 10% of values for indicators that were expected to fall, such as transferrin receptor. The change in values of these 10th or 90th percentiles was also expressed as SDUs. This analysis was based on the assumption that the most iron deficient subjects would show the greatest change as a result of the interventions. Table 1 shows how the indicators performed when assessed in these two ways.

An analysis of the effect of using different thresholds of success varying between 0.1 and 0.5 SDUs did not change the inferences about each indicator in each study. In the one study in which serum ferritin did not meet the criterion for success based on the change for all subjects, neither did any other indicator. The indicator of body iron stores calculated from serum ferritin and transferrin receptor did not perform better than serum ferritin alone. It was not possible to distinguish between the performance of haemoglobin, transferrin receptor, zinc protoporphyrin and mean cell volume.

Table 1 Success of indicators to detect changes in iron status in 10 controlled trials of treatments, estimated as the number of indicators showing a change of ≥ 0.2 standard deviation units (SDUs)

Indicator of iron status	Success of indicator based on mean change of ≥ 0.2 SDUs for all subjects	Success of indicator based on mean change of ≥ 0.2 SDUs for top or bottom 10% ^a
Haemoglobin	60%, 6 of 10 studies	80%, 8 of 10 studies
Mean cell volume	50%, 2 of 4 studies	75%, 3 of 4 studies
Serum ferritin ^b	90%, 9 of 10 studies	60%, 6 of 10 studies
Transferrin receptor ^c	56%, 5 of 9 studies	56%, 5 of 9 studies
Body iron stores	78%, 7 of 9 studies	78%, 7 of 9 studies
Zinc protoporphyrin	50%, 3 of 6 studies	67%, 4 of 6 studies

^a Depends on whether the indicator was expected to rise or fall.

^b Transformed to logarithms.

^c Results were the same with or without transforming values to logarithms.

The analysis was based on trials that had a control group to account for secular and random change. However, many programmes simply evaluate differences between before and after an intervention, and do not have a control group in the design. To examine whether different indicators would be selected in the absence of a control group, two questions were asked.

First, in how many studies did the value of the indicator change in the control group by ± 0.2 SDUs? A change of this size in either direction would imply that the indicator is not very stable. It could also indicate an additional source of iron other than the intervention, or a change in exposure to hookworms or malaria, that resulted in a change in iron status. Second, in how many studies would the wrong inference have been made if there had been no control group to account for secular or random changes?

Four studies were excluded from these analyses because they involved pregnant women or young children among whom changes would have been expected anyway, without any intervention. Of the remaining six studies, which were done among schoolchildren or non-pregnant women, mean cell volume was only measured in one study and zinc protoporphyrin in two. Table 2 shows the results of this analysis, which indicate that serum ferritin and body iron stores performed best.

Table 2 The results of an analysis of the stability of indicators of iron status in control groups during studies of interventions and of the inferences that would have been wrong without a control group

Indicator of iron status	Number of studies in which there was a significant change in the control group of ± 0.2 SDUs	Number of studies in which the inference would have been wrong without a control group
Haemoglobin	4 of 6	2 of 6
Serum ferritin	1 of 6	1 of 6
Transferrin receptor	3 of 6	2 of 6
Body iron stores	1 of 6	1 of 6
Zinc protoporphyrin	2 of 2	1 of 2

SDUs, Standard deviation units.

Based on results of the analysis presented in Table 1 and 2, it was concluded that serum ferritin is the indicator of choice to evaluate the impact of interventions to control iron deficiency in studies with or without control groups.

5.2 Performance of indicators to predict a change in haemoglobin concentration in response to iron intervention

A critical issue for the Consultation was to decide which current indicator represents the best means to identify a true iron deficiency and could act as the “gold standard” by which to evaluate alternative indicators. The change in haemoglobin concentration following intervention using iron was chosen based on the assumption that the size of any change was likely to be strongly related to the degree of iron deficiency. This measure has been used in previous studies (13). It has a disadvantage because if factors other than iron deficiency contribute to anaemia, such as a vitamin A deficiency, then the haemoglobin concentration will not respond to treatment with iron alone.

A linear regression analysis was performed to examine the degree to which baseline indicators of iron status predict a change in haemoglobin, using the following model:

$$Y = \beta_0 + \beta_1 * X_1 + \beta_2 * X_2 + \beta_3 * X_1 X_2 + \epsilon$$

Where: Y is the change in haemoglobin concentration from baseline to follow-up

X_1 is the group (control versus intervention)

X_2 is the baseline iron indicator measured in SDUs

$X_1 X_2$ is the interaction between group and iron indicator

ϵ is the error or residual.

The coefficient of interest in this analysis is (β_3) defined as the excess change in haemoglobin concentration for intervention over control for each additional SDU of the selected iron indicator at baseline. A statistically significant interaction term (β_3) was interpreted to mean that the indicator was associated with a change in haemoglobin concentration in response to intervention with iron, whether given as supplements or as fortified food. An indicator was arbitrarily classified as successful if there was an increase in haemoglobin concentration by ≥ 3 g/l for each SDU of the selected indicator at baseline. An advantage of this approach is that it uses the control group to take into account both secular trends in haemoglobin concentration and regression to the mean. Variables that were not normally distributed were transformed to logarithms to see if their predictive power could be improved.

Table 3 shows the success of each indicator in predicting the change in haemoglobin concentration in response to an iron intervention. Using an increase of 2–5 g/l made no difference to the results of the analysis.

Table 3 The success rate of indicators of iron status to predict an increase in haemoglobin of ≥ 3 g/l for each standard deviation unit of the indicator at baseline

Indicator of iron status	Success rate
Haemoglobin	10%, 1 of 10 studies
Mean cell volume	25%, 1 of 4 studies
Serum ferritin ^a	40%, 4 of 10 studies
Transferrin receptor	33%, 3 of 9 studies
Body iron stores	44%, 4 of 9 studies
Zinc protoporphyrin	17%, 1 of 6 studies

^a Transformed to logarithms.

Table 3 shows that the indicators were generally less successful in predicting the response of the haemoglobin concentration to treatment with iron than in assessing the response to interventions shown in Table 1. In fact in six of the ten studies, including the three studies in Africa, none of the indicators were associated with the change in haemoglobin concentration. This could have been because low haemoglobin concentrations were caused by factors other than iron deficiency, such as infectious diseases, which impaired the haemoglobin response to the interventions. Nevertheless, this analysis led to the following tentative conclusion, that serum ferritin or transferrin receptor are the best indicators to predict a change in haemoglobin concentration in response to iron intervention and, if both indicators are measured, then body iron stores can be estimated as well. But these indicators were successful in less than half of the studies analysed.

6. Indicators of inflammation

The Consultation considered that serum ferritin was the best indicator of the impact of an iron intervention as well as being a useful indicator of depleted iron stores. However serum ferritin is also an acute phase protein, which means that its concentration rises during inflammation, so the customary thresholds to indicate an iron deficiency of <12–15 µg/l may no longer apply. One way of dealing with this issue is to set the threshold higher, and a threshold of <30 µg/l has been recommended in the presence of infection, but only for children <5 years old (1). There is a need to examine the value of using different thresholds among infected older children and adults.

The Consultation proposed that the measurement of an acute phase protein could help to interpret data on serum ferritin: if the concentration of the additional acute phase protein is higher than the normal threshold it could indicate underlying inflammation and explain a high serum ferritin concentration in the presence of iron deficiency.

One way of controlling for a high serum ferritin concentration resulting from infection would be to use the concentration of another acute phase protein to exclude individuals whose measurements of both indicators are above a certain threshold. This approach is not considered feasible in many parts of sub-Saharan Africa where many people are infected with *Plasmodium* spp., the cause of malaria, and are either asymptomatic or have only mild disease and yet have high concentrations of acute phase proteins in their blood (14). Many of the same individuals may also be chronically infected with one or more species of worms, which may also contribute to an acute phase response as well as to anaemia and iron deficiency because of the blood loss they cause. There may even be an acute phase response without a loss of blood. Excluding individuals with

high concentrations of acute phase protein may, in circumstances in which repeated or chronic infections are common, reduce the sample size substantially and leave an atypical residual sample.

The Consultation felt that there was a need for the analysis of data on the relationship between serum ferritin, transferrin receptor and different acute phase proteins to assess which was best correlated with serum ferritin during different stages of infection. For example, it may be possible to control for high serum ferritin concentrations using one or more acute phase proteins. Several acute phase proteins could be used for this purpose including C-reactive protein (CRP), α -1-antichymotrypsin (ACT), α -1 acid glycoprotein (AGP), serum amyloid A, fibrinogen and haptoglobin. The most frequently used acute phase proteins are CRP, which responds quickly to inflammation but also subsides quickly in concentration; ACT which also rises quickly but remains at a high concentration longer than CRP; and AGP which is slower to respond than CRP or ACT but remains at a high concentration for longer than either (15,16). The concentration of AGP maybe a better indicator than CRP or ACT of the presence of chronic, sub-clinical infection, and may better reflect the changes in the concentration of ferritin during infections.

The Consultation proposed that data should be sought from studies in diverse settings that have, if possible, measured haemoglobin concentration, serum ferritin and transferrin receptor, and at least one acute phase protein, with CRP, ACT and AGP as the first choices. Because an assay for transferrin receptor has only relatively recently become available, data sets that do not include this measurement but have measured another acute phase protein as well as serum ferritin would also be useful. Data on the presence of infectious diseases or on malaria parasitaemia, and on the intensity of worm infections would also be helpful to examine the relationship between specific infections and acute phase proteins.

7. Recommendations

The Consultation made the following recommendations based on the analysis of studies presented, the literature reviews, and on the debates during the Consultation.

7.1 Assessing the iron status of populations

The concentration of haemoglobin should be measured, even though not all anaemia is caused by iron deficiency. The prevalence of anaemia is an important health indicator and when it is used with other measurements of iron status the haemoglobin concentration can provide information about the severity of iron deficiency.

Measurements of serum ferritin and transferrin receptor provide the best approach to measuring the iron status of populations. In places where infectious diseases are common, serum ferritin is not a useful indicator because inflammation leads to a rise in the concentration of serum ferritin as a result of the acute phase response to disease. If infectious diseases are seasonal, then the survey should be done in the season of lowest transmission. In general the concentration of transferrin receptor does not rise in response to inflammation so that, when combined with the concentration of serum ferritin, it is possible to distinguish between iron deficiency and inflammation. Table 4 indicates how data on serum ferritin and transferrin receptor may be interpreted based on the experience of participants of the Consultation. For the purposes of describing the prevalence of iron deficiency in a population with a single number, the prevalence based on serum ferritin should be used except where inflammation is prevalent (Table 4, row 2) in which case the prevalence based on transferrin receptor is more appropriate. However, the proposed classification still requires validation in population surveys.

Table 4 The interpretation of low serum ferritin and high transferrin receptor concentrations in population surveys: this classification is based on experience of measuring ferritin and transferrin receptor in research studies and requires validation in population surveys

Percentage of serum ferritin values below threshold ^a	Percentage of transferrin receptor values above threshold ^b	Interpretation
<20% ^c	<10%	Iron deficiency is not prevalent.
<20% ^c	≥10%	Iron deficiency is prevalent; inflammation is prevalent.
≥20% ^d	≥10%	Iron deficiency is prevalent.
≥20% ^d	<10%	Iron depletion is prevalent.

^a Apply thresholds by age group given in WHO, UNICEF, UNU (1).

^b Apply thresholds recommended by manufacturer of assay until an international reference standard is available (See Section 8, Priorities for research, below).

^c <30% for pregnant women.

^d ≥30% for pregnant women.

Studies are needed to determine the best procedures to process, transport and store biological samples in which transferrin receptor will be measured, and to establish internationally applicable thresholds to classify the iron status of populations.

It can be useful also to measure the concentration of an acute phase protein, if funding is available. The most commonly measured acute phase protein is CRP, but there is evidence that AGP may better reflect the change in concentration of ferritin in serum and may be the most useful acute phase protein to measure. A number of commercial assays are available for measuring these proteins but, except for CRP, there are no international reference standards available, resulting in reference ranges specific to each assay. In such circumstances, the threshold recommended by the manufacturer should be used.

7.2 Evaluating the impact of interventions to control iron deficiency in populations

Serum ferritin is the best indicator of a response to an intervention to control iron deficiency and should be measured with the haemoglobin concentration in all programme evaluations. In circumstances in which iron deficiency is the major cause of anaemia,

the haemoglobin concentration may improve more rapidly than the serum ferritin concentration. In circumstances in which the serum ferritin concentration improves (even when inflammation is common) but the haemoglobin concentration does not, factors in addition to iron are likely to be the cause of anaemia.

If funding is available, it could also be useful to measure the concentration of one or both of the acute phase proteins CRP or AGP, to account for a high serum ferritin concentration caused by inflammation. Individuals with high values for the acute phase protein should be excluded from the analysis, if possible, depending on the limitations imposed by the sample size of the dataset and the consequent translation of the results to define the iron status of the general population. This is particularly important when repeated surveys are done and there is no control group for the intervention.

If funding is available, the transferrin receptor should be measured during repeated surveys to classify populations according to the criteria shown in Table 4. The combination of serum ferritin and transferrin receptor may also be used to estimate body iron stores in populations (12). The calculation of body iron stores is not essential but can be useful to estimate the amount of iron that is absorbed during an intervention and to demonstrate a decrease in iron deficiency. However, since the method uses measurements of serum ferritin concentration, infection may again be a confounding factor, so an acute phase protein should be measured to exclude individuals with a high concentration.

A working group will be established to coordinate the analysis of data sets containing estimates of serum ferritin, transferrin receptor and acute phase proteins and to make suggestions about how to improve the assessment of iron status.

In three years time, another consultation will be held to evaluate the recommendations made here based on the results of recent studies of assessing iron status.

8. Priorities for research

- There is an urgent need for an international reference material with a certified concentration of transferrin receptor to standardize transferrin receptor assays.
- A review of existing data is needed to confirm the thresholds used in Table 4 to derive a classification of iron status. The analysis should also examine alternative approaches using serum ferritin alone, and with one or two acute phase proteins. Thresholds for other indicators of iron status, such as zinc protoporphyrin, should also be examined if possible.
- A review of existing data is needed to examine which acute phase proteins might best be used to interpret data on serum ferritin during both acute and chronic infections, and whether data on serum ferritin could be corrected rather than excluded. This review would identify the best acute phase protein to use and the thresholds to apply for both the acute phase protein and serum ferritin, in health and during infection.
- There is a need for simple instruments that can be used in the field to measure indicators of iron status such as ferritin, transferrin receptor or acute phase proteins, or simple methods to collect samples for analysis, such as dried spots of blood or serum.
- The thresholds and ranges for all indicators of iron status need to be defined and validated for children aged 6–24 months.
- Controlled studies are needed to further examine how body iron stores that have been estimated using the ratio between transferrin receptor and ferritin change in response to interventions to improve iron status.
- Additional iron intervention studies are required to assess the validity of the recommended indicators.

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11. List of participants

Dr Pierre Adou, Country Director, Helen Keller International, Nutrition Department, Abidjan, Côte d'Ivoire

Ms Maria Andersson, Technical Officer, Department of Nutrition for Health and Development, World Health Organization, Geneva, Switzerland

Dr Bruno de Benoist, Medical Officer, Department of Nutrition for Health and Development, World Health Organization, Geneva, Switzerland

Professor John Beard, Department of Nutrition Science, Pennsylvania State University, University Park, PA, USA

Dr Jacques Berger, Senior Researcher, Institut de Recherche pour le Développement (IRD), Montpellier, France/Hanoi, Viet Nam

Dr Francesco Branca, Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione, Rome, Italy

Professor Gary Brittenham, Columbia University Medical Center, New York, NY, USA

Professor Chunming Chen, Chinese Center for Disease Control and Prevention, Beijing, China

Dr Christine Clewes, International Micronutrient Malnutrition Prevention and Control (IMMPaCt) Program, Centers for Disease Control and Prevention, Atlanta, GA, USA

Dr Mary Cogswell, Division of Nutrition and Physical Activity, Centers for Disease Control and Prevention, Atlanta, GA, USA

Professor James Cook, Department of Medicine, University of Kansas, Kansas City, KS, USA

Dr Lena Davidsson, (formerly Swiss Federal Institute of Technology), Nutritional & Health-Related Environmental Studies Section, International Atomic Energy Agency, Vienna, Austria

Dr Ines Egli, Technical Officer, Department of Nutrition for Health and Development, World Health Organization, Geneva, Switzerland

Dr Wilma Freire, Chief, Nutrition Unit, WHO Regional Office for the Americas, Pan American Sanitary Bureau, Washington, DC, USA

Dr Laurence Grummer-Strawn, Branch Chief, Maternal and Child Nutrition, Division of Nutrition and Physical Activity, Centers for Disease Control and Prevention, Atlanta, GA, USA

Dr Andrew Hall, University of Westminster, London, UK (*Rapporteur*)

Dr Eva Hertrampf, Institute of Nutrition and Food Technology (INTA), University of Chile, Santiago, Chile

Professor Lena Hulthén, Institute of Internal Medicine, Sahlgren Academy at Göteborg University, Sahlgren University Hospital, Göteborg, Sweden

Professor Richard Hurrell, Laboratory for Human Nutrition, Institute of Food Science and Nutrition, Swiss Federal Institute of Technology, Zurich, Switzerland

Dr Sa'ad Kharabsheh, Secretary General, Ministry of Health, Amman, Jordan

Professor Prakash Kotecha, Preventive and Social Medicine, Government Medical College, Vadodara, India

Professor Sean Lynch, Department of Clinical Medicine, Eastern Virginia Medical School, Norfolk, VA, USA

Dr Zuguo Mei, Division of Nutrition and Physical Activity, Centers for Disease Control and Prevention, Atlanta, GA, USA

Dr Nguyen Xuan Ninh, Senior Researcher, Micronutrient Research Department, National Institute of Nutrition, Hanoi, Viet Nam

Professor Omar Obeid, Department of Nutrition and Food Science, American University of Beirut, Beirut, Lebanon

Dr Ibrahim Parvanta, Chief, International Activities, Maternal and Child Nutrition Branch, Division of Nutrition and Physical Activity, Centers for Disease Control and Prevention, Atlanta, GA, USA

Dr Christine Pfeiffer, Chief, Nutrition Laboratory, Division of Laboratory Science (DLS), National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, USA

Professor Rebecca Stoltzfus, Division of Nutritional Sciences, Cornell University, Ithaca, NY, USA (*Chairperson*)

Dr Anna Verster, WHO Senior Technical Advisor, Global Alliance for Improved Nutrition, Geneva, Switzerland

Dr Salvador Villalpando, Instituto Nacional de Salud Publica, Center for Research on Nutrition and Health, Cuernavaca, Mexico

Ms Trudy Wijnhoven, Technical Officer, Department of Nutrition for Health and Development, World Health Organization, Geneva, Switzerland

Professor Pattanee Winichagoon, Deputy Director for Academic Affairs, Institute of Nutrition, Department of Community Nutrition, Mahidol University, Nakhom Pathom, Thailand

Professor Mark Worwood, Department of Haematology, College of Medicine, University of Wales, Cardiff, Wales

Dr Mark Young, Senior Health Adviser, Health Section, United Nations Children's Fund, New York, NY, USA

Appendix 1

The main biochemical indicators of iron status

Measurement	Sample	Commonly used methods*	Units	Indicator of	Advantages	Disadvantages
Bone marrow iron (haemosiderin)	Bone marrow aspirate	Microscopical examination of stained marrow cells	Semi-quantitative grading	Depleted or absent body iron stores	Indicates body iron stores and correlates well with other indicators	Invasive and traumatic to collect sample
Haemoglobin	Whole blood	Cyanmethaemoglobin using colorimeter or spectrophotometer or azide-methaemoglobin using e.g. HemoCue®	g/l	Anaemia	Simple to measure; important functional and public health consequences	Anaemia occurs without iron deficiency; adjustment of thresholds needed for age, sex, pregnancy, altitude, smoking and some ethnic groups
Haematocrit or packed cell volume (PCV)	Whole blood	Centrifugation of whole blood in capillary tube or value derived from automated flow cytometry	Decimal ratio or %	Proportional volume of RBCs in whole blood	Simple to measure	Same as haemoglobin; depends on factors affecting centrifuge e.g. stable power supply
Mean cell volume (MCV)	Whole blood	Calculated from haematocrit and RBC count using haemocytometer, or value derived from automated flow cytometry	fl (10 ⁻¹²)	Average RBC size: low is microcytic; high is macrocytic	RBC index. Average size of RBCs can be characteristic of type of anaemia	Requires expensive machine to be reliable; low in thalassaemia and inflammation
Mean cell haemoglobin (MCH)	Whole blood	Haemoglobin concentration and RBC count using haemocytometer, or value derived from automated flow cytometry	pg (10 ⁻⁹)	Haemoglobin in an average RBC; if low, hypochromic; if normal, normochromic	As for MCV	Requires expensive machine to be reliable; slow to respond to iron deficiency

Measurement	Sample	Commonly used methods ^a	Units	Indicator of	Advantages	Disadvantages
Red cell distribution width (RDW)	Whole blood	Automated flow cytometry calculates $RDW = \frac{\text{Standard deviation of MCV}}{MCV}$	%	Abnormal range in size of RBCs <11.5% or >14.5%	Size distribution of RBCs can be characteristic of type of anaemia	Requires expensive machine to be reliable; high in iron deficiency; low in thalassaemia and inflammation
Reticulocyte haemoglobin concentration	Whole blood	Automated flow cytometry	g/l reticulo-cytes	Concentration of haemoglobin in new RBCs	Represents new RBCs 18–36 hours old, thus recently affected by deficiency	Requires expensive machine to be reliable
Serum or plasma iron	Serum or plasma (not using EDTA)	Colorimetry	µg/dl µmol/l	Iron bound to transferrin in blood	Measure of iron supply to the bone marrow and other tissues	Varies diurnally and after meals; sample easily contaminated with iron from outside sources; low in chronic disease
Erythrocyte protoporphyrin	Whole blood or dried blood spots	Usually estimated from ZPP (below); expressed as ratio to haemoglobin concentration	µg/dl whole blood or RBCs	Restricted supply of iron to developing RBCs	Useful in young children; whole blood or dried spots can be assayed	Increased in iron deficiency, inflammatory disorders, exposure to lead
Zinc protoporphyrin (ZPP)	Whole blood or dried blood spots	Fluorescence spectrophotometry or portable Aviv@ haematofluorimeter	µmol/mol of haemo-globin	Lack of iron to developing RBCs	Useful in young children; whole blood or dried spots can be assayed	Increased in iron deficiency, inflammatory disorders, exposure to lead
Ferritin	Serum or plasma	Immunoassay e.g. enzyme-linked immunosorbent assay (ELISA) or immuno-turbidometry	µg/l	Size of iron stores	Reflects iron status	Ferritin is an acute phase protein so concentration is increased in inflammatory disease and sub-clinical infection
Total iron binding capacity (TIBC)	Serum or plasma	Colorimetric assay of amount of iron that can be bound to unsaturated transferrin <i>in vitro</i> ; determination from transferrin concentration measured immunologically	µg/dl µmol/l	Total capacity of circulating transferrin bound to iron	Increased in iron deficiency, low in inflammatory disorders	Large overlap between normal values and values in iron deficiency

Measurement	Sample	Commonly used methods ^a	Units	Indicator of	Advantages	Disadvantages
Transferrin saturation	Serum or plasma	Calculated from: $\frac{\text{Serum iron}}{\text{TIBC}}$	%	Saturation of <15% with high TIBC indicates iron deficiency	Proportion of transferrin bound to iron	Same as for serum iron
Transferrin receptor	Serum or plasma	Immunoassay e.g. ELISA or immunoturbidometry	µg/l	Reflects balance between cellular iron requirements and iron supply	Semi-quantitative measure of the severity of iron deficiency even in presence of inflammatory disorders	Affected by the rate of erythropoiesis
Body iron stores	Serum or plasma	Ratio of transferrin receptor to ferritin $-\frac{[\log_{10}(\text{TfR}/\text{ferritin ratio}) - 2.8229]}{0.1207}$	mg/kg	Measure of body iron status including iron deficits, status of storage iron and iron overload	Measure of full range of iron status, validated by phlebotomy studies in adult volunteers	Same limitations as component parts
Hepcidin	Serum or plasma, urine	Immunoassay for pro-hepcidin e.g. ELISA	ng/ml	Regulator of iron absorption from gut	Production diminished when iron reserves depleted	Assay methods and interpretation of results is under development

RBC, red blood cell.

^a The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization or the Centers for Disease Control and Prevention in preference to others of a similar nature that are not mentioned.

^b Cook, Flowers, Skikne (12).

Appendix 2

Selected biochemical indicators of iron status and an acute phase protein with common methods of measurement, cost, variability, thresholds and reference material

Indicator	Common method or equipment ^a	Approximate cost of equipment US\$	Cost/test of supplies and materials US\$	Minimum volume for one analysis	Complexity	Sampling/biological variability ^b	Threshold	Reference material
Haemoglobin	HemoCue® photometer	400	<1	50 µl whole blood	Low	Low/low	110–130 g/l ^c	WHO
Zinc protoporphyrin	Haemato-fluorimetry e.g. AVIV® hematofluorimeter	5 000	1	50 µl whole blood	Low	Medium/medium	>70–80 µg/dl red blood cells ^c	Not available
Mean cell volume	Particle counter e.g. Coulter® counter	15 000	5	300 µl whole blood	Low	Low/low	<67–81 fl ^c	Not available
Transferrin receptor	Immunoassay e.g. ELISA	5 000	10–15	100 µl serum or plasma	Medium	Medium/medium	Not defined ^d	Not available
Ferritin	Immunoassay e.g. ELISA	5 000	5–10	100 µl serum or plasma	Medium	Medium/medium	<12–15 µg/l ^c	WHO

C-reactive protein	Immunoassay e.g. ELISA Orion® QuickRead	5 000 3 000	8 10	50 µl serum or plasma 20 µl serum or plasma	Medium Low	Medium/ medium Medium medium	<3–10 mg/l <5 mg/l	IFCC ^e IFCCe
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ELISA, enzyme-linked immunosorbent assay.

^a The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization or the Centers for Disease Control and Prevention in preference to others of a similar nature that are not mentioned.

^b Sampling variability depends on operator training.

^c WHO, UNICEF, UNU (7).

^d Use thresholds recommended by manufacturer of assay.

^e International Federation of Clinical Chemistry.