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Proposed recommendations for measurement of serum iron in human blood

An expert panel on iron[1] was convened by the International Committee for Standardization in Hematology at its meeting in Sydney, Australia, in August 1966. The investigations undertaken by the panel were described in a symposium at the XIII Congress of the International Society of Haematology in Munich in August 1970 (to be published). On the basis of the report submitted by the panel to the Board, ICSH proposes the following recommendations for the preparation of a reference material and a reference method for serum iron assay.

It is not intended at this time to manufacture and issue an international reference preparation but national committees or standards organizations are requested to ensure that reference preparations manufactured under their jurisdiction conform to the recommendations.

Comments on these proposals and requests for further information should be submitted to the Chairman of the Panel (Dr J. Fielding, St. Mary's Hospital, Harrow Road, London, W9, UK) or to the ICSH Secretariat (c/o Dr S. M. Lewis, Royal Postgraduate Medical School, London, W12, UK).

1 Material for Serum-iron Reference Preparation

1.1 The material should consist of sterile pooled human sera obtained from normal subjects, taken and handled at all steps of processing with sterile techniques.

1.2 Presently, it appears that horse serum may be substituted for human serum.

1.3 The haemoglobin concentration of the serum pool should not exceed 3 mg per 100 ml (10 μg per 100 ml of haemoglobin iron).

1.31 These limits of haemoglobin contamination are difficult to achieve without removing haemoglobin from the serum pool by methods such as ultrafiltration. However, they can be attained if care is exercised in the bleeding of donors and the separation of the serum from the blood clot. The following method is recommended:

Bleeding should be accomplished with a thin-walled 16 gauge hypodermic needle with free flow of the blood into iron-free polyethylene plastic bottles. Keep the blood at room temperature for about 16 hours. Ring the clot carefully and spin the specimen at 2,500 rpm for 45 minutes. Decant the serum carefully to avoid contamination with red blood cells. Recentrifuge the serum and decant the serum from red blood cells a second time. Haemoglobin concentration should be measured by a method as sensitive as the benzidine-peroxide technique.

1.4 The serum pool should contain no iron chelators other than those which are native to the serum.

1.41 The presence of iron chelators can be detected by adding a known amount of iron to an aliquot from the serum pool and showing a slowed rate of colour development with iron chromagens when compared with colour development in similarly treated aqueous solutions or normal serum.

1.5 Human serum should be obtained from subjects who were fasted overnight. This point is not relevant to horse serum.

2 Preparation of Serum-iron Reference Preparation

2.1 Two lots of serum of equal volume should be taken from the serum pool. To one lot add 1·00 ml of an iron standard solution containing a weighed amount of iron wire (1·00 mg per ml) for each litre of serum. A recommended method for preparing standard iron solutions is provided in section 3. To the other lot of serum add an equal volume of iron-free distilled water.

[1]The expert panel consists of the following members:
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2.2 Distribute measured volumes of serum from each lot into iron-free containers. The volume of serum in each container should not be less than 10 ml. The specimens should be lyophilized to dryness.

2.3 The containers should be labelled to include the following data:

2.31 The volume of iron-free water required to reconstitute the specimen to its original volume.

2.32 The iron concentration of the reconstituted material as certified by designated reference laboratories.

2.33 The expiration date of the specimen when stored at 4°C one year from manufacture.

2.34 The origin of the specimen and appropriate lot identification.

3 ICSH Reference Method for Assay of Reference Preparations

3.1 The following methods for the preparation of materials for use in iron analysis are recommended:

3.11 Iron-free water should be prepared by passing distilled water through a deionizer which contains both charcoal and ion exchange resin.

3.12 Iron-free glassware may be prepared by soaking Pyrex glassware for six hours in HCl (2 mol/l) or sulphuric acid-sodium dichromate cleaning solution and rinsing it free from acid in repeated changes of iron-free water. Certain plastic containers are supplied by the manufacturer in an iron-free condition. Surveillance must be maintained over new lots of plastic to ensure it is not contaminated with iron.

3.13 Iron standard solution should be prepared from a concentrated iron stock solution. The iron stock solution may be made by placing 0·100 g of dry, polished certified electrolytic iron wire in 2 ml of redistilled hydrochloric acid, iron-free (7 mol/l). The container should be placed in a boiling water bath to bring the wire into solution. Then the acid iron solution should be cooled and brought to 100 ml with iron-free water. Iron standard solutions containing 2 μg per ml of iron can be made by adding 2 ml of the iron stock solution to a 1 litre volumetric flask and bringing the volume to 1,000 ml with redistilled hydrochloric acid (0·005 mol/l).

3.2 The following method is recommended for measurement of the iron concentration as a reference method.

3.21 Reagents include three solutions:

(*a*) Protein precipitant: aqueous solution made to contain 100 g trichloroacetic acid (redistilled); 30 ml thioglycollic acid and 2 mol hydrochloric acid (redistilled) per litre. This should be stored in a dark brown bottle, and under these conditions is stable for at least two months.

(*b*) Chromagen solution: sodium acetate (2 mol/l) containing 250 mg bathophenanthroline sulfonate per litre.

(*c*) Iron standard solution: 2 μg of iron per ml.

3.22 The test procedures must be performed using iron-free test tubes:

(*a*) To 2 ml of serum add 2 ml of the protein precipitant solution. Mix thoroughly, eg, with a vortex mixer; let stand for five minutes and centrifuge to an optically clear supernatant.

(*b*) To 2 ml of iron standard solution add 2 ml of the protein precipitant solution. Mix vigorously, eg, with a vortex mixer; let stand for five minutes.

(*c*) Prepare a reagent blank by substituting iron-free water for serum as described in (*a*).

(*d*) To 2 ml of the supernatant solution in (*a*) and in the mixtures (*b*) and (*c*), add 2 ml of the chromagen solution. Mix each tube thoroughly and let stand for at least five minutes.

(*e*) Measure the optical density of the chromagen-treated solutions in a spectrophotometer with wavelength set at 535 nm after appropriate calibration of the instrument with a cuvette containing distilled water.

(*f*) Calculate the iron concentration of the serum specimen from the formula:

$$\text{Serum specimen } (\mu g/100 \text{ ml}) = \frac{\text{OD serum specimen} - \text{OD blank}}{\text{OD standard} - \text{OD blank}} \times 200.$$

The OD blank should not exceed 0·015 against distilled water in a 1 cm pathway.

4 Independent Methods for Serum-iron Assays by Reference Laboratories

4.1 Reference laboratories should be encouraged to measure the iron concentration in reference materials by independent methods such as wet digestion techniques or atomic adsorption spectroscopy. Values obtained by these methods require correction for the haemoglobin-iron concentration of the reference materials (1 g haemoglobin contains 3·47 mg iron).

5 Recommended Routine Method for Serum-iron Assay

We recommend the reference method for serum-iron assay above as a suitable clinical method. The panel has no experience of using smaller volumes than those indicated, but sees no *a priori* reason why these should not be scaled down if convenient.