

SECTION III

PROCEDURE FOR SERUM IRON ESTIMATION

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CHAPTER 14

INDISPENSABLE PRE-REQUISITES FOR SERUM IRON ESTIMATION

Estimation of Serum Iron is a very delicate investigation in which the possibilities of iron contamination are many more than what is generally realised. Therefore, great care is necessary to be exercised to prevent iron contamination during collection of blood samples, preparation of reagents, preparation of glassware, preparation of iron standards and during the actual estimation of iron in serum.

Another important aspect is the proper selection of subjects. This is important particularly for the determination of normal serum iron values. Serum iron values are influenced by great majority of factors. All these factors should, therefore, be considered while selecting the subjects and collecting the blood samples. In order to fulfil the desire of achieving consistent results, whole procedure should be carried out under the standard laid down in conditions.

PRECAUTIONARY MEASURES

- (1) The test tubes containing Blank, Standards and Test Samples should be closed or plugged with non-absorbable cotton except during the time of adding the reagents. This is to prevent the dust particles falling into the tubes.
- (2) The glass windows should preferably be kept closed.
- (3) Fan should not be kept working.
- (4) To prevent the patient from seeing amount of blood collected for serum iron estimation, his/her eyes should be covered. If this is not done, it would lead to the mental upset on the part of patient/s, loss of confidence in the treating doctor and at times absconding of patient/s too. A healthy co-operation of the patients is highly essential for the work to be continued.
- (5) The patients should be called to Laboratory for collection of blood samples for serum iron estimation. This is to be done for the following purposes :
 - (a) To prevent the contamination of "iron-free" glassware with the atmospheric dust. This is of particular importance in breezy or rainy atmospheric conditions.
 - (b) To prevent other patients in the ward from seeing the amount of blood collected.

SERUM IRON IN NORMAL SUBJECTS

It is highly desirable that each investigator should have his own standards. Hence, the determination of serum iron values in Normal Subjects (Normal Adult Males and Normal Adult Females), with the method adopted for the serum iron estimation, and also in the type of subjects selected for the study, is essential. Serum iron values were, therefore, estimated in 40 normal healthy adults, out of which 20 were males and 20 were females.

SELECTION OF NORMAL SUBJECTS

This aspect is most important so far as Estimation of Serum Iron values in normal subjects is concerned. Forty normal adults, with equal number of males and females, were selected for the purpose. They were all non-anaemic and otherwise normal. They gave no history of any recent illness and were physically fit. The blood samples were collected in the morning between 6-0 a.m. and 7-0 a.m. as a rule, with the subjects in the fasting state (as in case of anaemic subjects). The following requirements must be satisfied :-

- (1) Age : Adults
- (2) Sex : Either
- (3) Haemoglobin concentration within normal limits
- (4) No history of any past illness a month preceding the investigations.

(5) Prescribed Medication :

There should be no history of taking or having taken any drugs, particularly iron preparations, drugs containing iron, and salicylates, during last 15 days.

(6) Self Medication :

There should be no history of taking or having taken Salicylates during last 15 days. In the present study, there were fair number of subjects who gave a definite history of taking Salicylates very frequently for headache and likewise symptoms. Such subjects were discarded from the study.

(7) Female Subjects:

They deserve a specific mention. The samples in female subjects were collected during the last week of menstruation in order to avoid, as far as possible, the variations due to the menstrual blood loss so that the serum iron values in normal adult males and normal adult females can be correctly compared.

SELECTION OF ANAEMIC SUBJECTS

The selection of Anaemic subjects (Male Adult patients and Female Adult patients of iron deficiency anaemia) has already been discussed in Chapter 9.

COLLECTION OF BLOOD SAMPLES

(1) A SEPARATE set of all-glass syringes and stainless steel needles for collecting blood samples for serum iron estimation is essential. Syringes and plain bulbs must be completely dry to prevent haemolysis of R.B.Cs.

(2) The amount of iron present in serum is very small as compared with that in erythrocytes. Hence, haemolysis must be avoided. Samples showing haemolysis are considered unsuitable and must be discarded.

(3) Blood samples were collected on the previous day and serum iron estimation was conducted on the next day because the time available after collecting the last (eight hours after the drug) sample falls short in completing the lengthy and time-consuming procedure for serum iron estimation on the same day.

(4) 10 c.c. of blood was collected from a suitable vein and was transferred to a "iron-free" plain bulb. The bulb was corked immediately and was allowed to stand at room temperature for about 1 to 2 hours, in order to allow the serum to separate. Then the bulb was transferred and stored in refrigerator till the onset of actual procedure for iron estimation on the next day.

(5) Time of collection of Blood Samples :

Blood samples were collected as a rule in the early

morning between 6-0 a.m. and 7-0 a.m. with the patients in fasting state. So was true for normal subjects too. Thus, the blood samples from normal subjects as well as from anaemic subjects were collected at identical time and under standard conditions in order to avoid variations due to variable factors like, diurnal variations, variations due to the ingested food material etc.

IRON STANDARDS

Maximum accuracy is necessary in preparation of standards particularly in weighing the Ferric Ammonium sulphate (A.R.) because the accuracy of co-relation, between the optical density and the iron concentration in microgram per 100 ml., ~~with~~ which directly affects the results of all serum iron investigations, depends on the accuracy of iron standards.

REAGENTS

- (1) All the reagents must be prepared from chemicals of the highest available purity, (free from iron-contamination).
- (2) All the reagents should be tested to confirm their freedom from iron as impurity.
- (3) Maximum care should be exercised during the preparation of reagents, to avoid any iron contamination.
- (4) All the reagents should be made up in aqueous solution and should be stored in pyrex (or plastic) reagent bottles with dust-proof flat glass stoppers.

WATER

Iron-free water (triple-glass distilled water) must be used all throughout for any purpose e.g. preparations of standards, preparation of reagents, preparation of glassware etc. The bottles of triple-glass-distilled water should be labelled properly lest use of other water should result in repetition of the whole set of investigation as indicated by the development of strong colour in the Blank.

GLASSWARE

All the glassware used for serum-iron estimation must be previously made iron-free, and completely dry. The glassware should preferably be of "Pyrex" grade to prevent the frequent breakage resulting in repetition of particular set of investigation and simultaneous economical loss.

The reasons of frequent breakage in case of ordinary type of glassware^{as} against ~~against~~ the 'Pyrex' variety are :

- (a) Chemical factor (prolonged immersion in HCl).
- (b) Physical factor (high temperature to ensure complete drying).
- (c) Mechanical factor (centrifugalisation etc.).
- (d) Repeated handling (particularly during preparation of glassware).

The syringes used for serum iron estimation must be made up of all-glass and needles of stainless steel. The use

of ALL-GLASS syringes and stainless^{steel}/~~needles~~ plays an important role in preventing iron contamination of blood during the collection of blood samples.

USE OF PIPETTES

(1) The amount of various reagents to be added during the process of serum iron estimation, is very small, and therefore demands great accuracy. Hence a labelled pipette must be kept SEPARATE for each of the reagents.

(2) The readings from the pipette (and also burette) are taken at the lower meniscus (for wetting materials) (upper meniscus for non-wetting materials).

(3) A separate dry "iron-free" pipette^{should be kept}/~~for~~ each of the Test samples of serum and so also for the aliquotes of the supernatants obtained after the precipitation of proteins. Thus, a pipette once used must be discarded.

(4) The pipette should never be dipped directly into the reagent bottles. The reagents are to be taken out in Test Tubes from reagent bottles and from the test tubes, the reagents are sucked into the pipette. The remaining portion of reagent in the test tube is to be discarded. This is practised because if the pipette is dirty or chemically impure, the whole lot of reagents becomes so.

(5) Graduated pipettes :

The fluid is not ~~to~~ to be blown out but the gravity should be allowed to act. At the end of delivery of the contents of the pipette, the tip of the pipette is allowed to touch the wall of the test tube and this is enough therefor.

(6) Volumetric pipettes:

At the end of delivery of the contents of the pipette, the bulb of the pipette is covered in first for $\frac{1}{2}$ - 1 minute, and this heat is enough to deliver the remaining amount of the fluid. Blowing is to be avoided to prevent contamination with saliva.

USE OF CUVETTES

(1) The outer surface of the cuvettes must be clean^{ed}/with a clean linen piece before taking the readings in Beckman spectrophotometer.

(2) In between taking the readings of two different samples, the cuvettes must be thoroughly washed with triple-glass-distilled water and then dried.

USE OF BECKMAN SPECTROPHOTOMETER

The instrument must be adjusted to zero by using a distilled water Blank. Then first set of readings of contents of all the 4 cuvettes was taken. Then, once again, the

instrument was adjusted to zero, and then the second set of readings was taken in the same way.

IDENTIFICATION OF THE SOURCE OF IRON CONTAMINATION

After careful establishment of the whole set up, the first set of test (unknown) samples of sera are collected with all precautions and iron is estimated. One Blank (B) and two standards (S_1 and S_2) are always included in each set of experiment. Development of colour in Blank or higher values of standard 1 and standard 2 are the Indicators for any iron contamination.

Failure to develop any colour whatsoever in the Blank and normal values of S_1 and S_2 rule out the possibilities of even doubting the purity (so far as the iron contamination is concerned) of the reagents. Hence, in subsequent investigation, colour development in Blank or higher values of S_1 and S_2 could be due to more common factor, like improper cleanliness of glassware or less commonly contaminated distilled water. Then a search should be made to find out the possible source of iron contamination and the test samples should be repeated for serum iron estimation.

At times, if there is slightest disturbance in the delicate instrument like spectrophotometer, it might start giving wrong readings. This again, could be judged from the readings of S_1 and S_2 and thence the necessary steps might be taken.

All the foregoing facts disclose the importance of inclusion of Blank (B) and two standards (S_1 and S_2) in each set of experiment.