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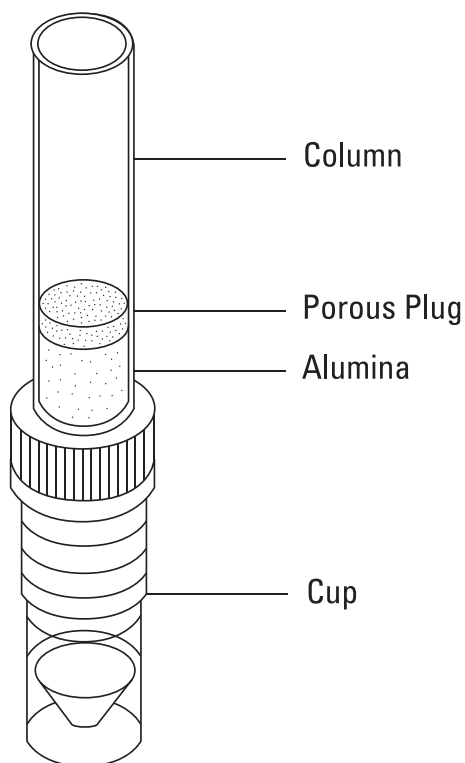
## NERL IRON SATURATING REAGENTS

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For use in the determination of total iron-binding capacity in serum

**MICRO COLUMN** (for use with 0.3 mL or 0.5 mL of serum)

Product No. ION 26002



### **SUMMARY AND EXPLANATION OF THE TEST**

The Iron-Binding Capacity (IBC) test (also known as Total Iron-Binding Capacity (TIBC) test) is used to determine the total ability of serum to combine with excess ferric ions. The iron-binding protein in serum is known as siderophilin or transferrin, and normally is only partially filled with iron (in the form of ferric ions). The IBC test measures the total amount of iron which transferrin is capable of binding.

### **PRINCIPLE**

A sample of patient's serum is mixed with a solution containing excess ferric ion which binds to unoccupied sites on transferrin. The mixture is passed through an alumina column which removes unbound ferric ion. Remaining (bound) ferric ion is determined by an appropriate method (not provided). This represents TIBC, and in calculating its value, correction must be made for the threefold dilution of serum by the iron solution.

### **REAGENTS**

Fifty prefilled disposable alumina columns, each sufficient for one determination and each containing a quantity of specially treated basic aluminum oxide equivalent to 300 mg of standard aluminum oxide.

One bottle (60 ml) of Iron Saturating Reagent (ferric chloride solution containing not less than 5 mg Fe<sup>+++</sup> per liter in 0.001 M citric acid with preservatives and stabilizers).

Reagents for the determination of iron are also required, but are not provided.

### **Warning and Precaution**

For in-vitro diagnostic use only.

### **Storage Instructions**

Store at ambient temperature (15° - 30°C) out of direct sunlight. Do not open foil envelopes containing columns until ready for immediate use.

### **Indications of Instability**

Do not use solution if it shows signs of contamination such as turbidity.

### **SPECIMEN COLLECTION AND PREPARATION**

Follow instructions pertaining to the method for determination of iron which is to be used. Serum, not plasma, should be used for this determination. Chelating agents may interfere. Patient samples should be drawn in the morning and in the fasting state. The serum should be separated rapidly from the cells. Serum may be stored up to 6 hours at room temperature and up to 3 days at refrigerated temperature (2° - 8°C). The column treated sample should be assayed immediately.

### **RECOMMENDED PRELIMINARY PROCEDURES**

**Control Sera:** On a daily basis, determine the TIBC value of a set of control sera of known TIBC content. The result obtained should fall within acceptable limits determined by the laboratory. This procedure may be combined with the quality control of the method for iron determination.

### **Additional Materials Required**

Pipette: 0.50 +/- 0.01 ml  
Dispenser or volumetric pipette: 1.00 +/- 0.02 ml  
Disposable plastic or glass tubes: 12 x 75 mm or larger  
Reagents and materials for determination of total iron

### **TEST PROCEDURE: MICRO COLUMN**

#### **Preliminary Comments**

Do not use reagents after their expiration date or if there are any indications of instability.  
(See indications of instability.)

#### **Test Procedure**

This test procedure may be performed using either a 0.3 mL sample volume or a 0.5 mL sample volume. It is recommended to run controls using the same sample volume as patient samples.

Select **ONE** of the following dilution methods.

a) In a clean disposable test tube, place 0.5 mL of serum to be analyzed and 1.0 mL of Iron Saturating Solution.

**OR**

b) In a clean disposable test tube, place 0.3 mL of serum to be analyzed and 0.6 mL of Iron Saturating Solution.

**Regardless of which sample method was chosen, continue with procedure as follows:**

- Mix by gentle inversion.
- Allow to stand for 3 to 5 minutes.
- Pour the serum mixture into the column.
- After about 8 minutes the mixture will have passed through the column and should be assayed immediately.
- Pass entire mixture through the column before removing a sample for iron assay.

### **RESULTS**

Follow instructions accompanying the reagents for iron determination. Be sure to include in your calculations, a factor of three, to account for the threefold dilution of the serum by the Iron Saturating Reagent.

### **LINEARITY**

Refer to the linearity range of iron channel in instrument operator's manual.

### **LIMITATIONS OF THE PROCEDURE**

The Iron Saturating Reagent contains many times the maximum amount of ferric ion needed to saturate the most unsaturated sera reported. The alumina in each column is more than enough to absorb all the iron in the reagent. Precise particle control of the alumina prevents the error caused by leaking of "fines", which has been reported for the magnesium carbonate procedure.

### **PERFORMANCE CHARACTERISTICS**

Blank determinations using distilled water (no serum) consistently give values of zero for iron content. Fifty sera run by the column procedure and a magnesium carbonate procedure gave a correlation coefficient of 0.99 with a least squares regression equation for the column procedure equal to 1.12 (magnesium carbonate value) -0.98. Between-run precision for a low-level serum pool gave a mean value of 242.7 µg/dl (N=20) with an SD of 7.3 and CV of 3.0%; a high pool gave a mean of 395.4 µg/dl, SD 9.8 and CV of 2.5%.

### **EXPECTED VALUES**

Normal values for TIBC will vary with population and the method used to determine iron in the treated and untreated samples. It is recommended that each laboratory determine the normal range for its particular population.

### **REFERENCES**

1. Henry, R.J. "Clinical Chemistry Principles and Techniques." Harper and Row, New York, NY (1974).
2. Tietz, N.W. "Fundamentals of Clinical Chemistry." W.B. Saunders, Philadelphia, PA (1976).