SimulTRAC* Radioassay Kit Vitamin B$_{12}$ $[^{57}\text{Co}]/\text{Folate}^{[125}\text{I}]}$

Catalog No. 06B262218 - 100 Tubes
Catalog No. 06B262226 - 200 Tubes
RIA HOT LINE U.S. ONLY: 1-800-431-1237

Customer support and technical information can be obtained at local ICN Pharmaceuticals offices

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SimulTRAC* Radioassay Kit Vitamin B$_{12}$ [$^{57}$Co]/Folate [$^{125}$I]

For Simultaneous Quantitative Determination of Vitamin B$_{12}$ and Folate in Serum and Plasma

Summary and Explanation of the Test

Lack of adequate tissue levels of vitamin B$_{12}$ or folate is the cause of megaloblastic anemia secondary to vitamin deficiency. Both vitamins are tightly protein bound in tissue, and serum blood levels reasonably predict total body stores.

Folate (folic acid) deficiency is observed in about one-third of all unsupplemented pregnant women, a majority of alcoholics and people whose diets are devoid of raw fruits and vegetables or fresh fruit juices. In addition, folate deficiency is associated with structural or functional damage in the upper third of the small bowel, with chronic hemolytic anemia, and in a number of other situations. Because saturated stores of total body folate are small and must be continuously replenished, serious deficiency may occur in weeks to months after intake is interrupted. Low serum levels of folate will precede, by several weeks, the appearance of megaloblastic changes. Depressed folate levels should be considered in patients with signs of megaloblastic anemia, as well as patients with the above clinical syndromes.

Vitamin B$_{12}$ deficiency is most frequently associated with pernicious anemia, gastric damage, distal intestinal damage and pure vegetarianism. Because of the very large tissue stores of B$_{12}$, years of dietary deficiency are necessary to deplete the body of this vitamin. Measurement of the serum level of "true" cobalamin, vitamin B$_{12}$ exclusive of analogues of vitamin B$_{12}$, is the definitive test for the diagnosis of vitamin B$_{12}$ deficiency and for evaluating patients with megaloblastic anemia or the aforementioned clinical syndromes.

For several reasons, it is advisable to determine serum vitamin B$_{12}$ and serum folate. Deficiencies of both may coexist. Morphologic alterations of the blood associated with vitamin B$_{12}$ deficiency are also produced by folic acid deficiency, and determination of the serum cobalamin and folate levels are necessary to adequately determine the cause of megaloblastosis. Treatment with folate in the face of B$_{12}$ deficiency may precipitate subacute combined degeneration of the spinal cord. Primary vitamin B$_{12}$ deficiency blocks the ability of cells to take up folate, thus providing a low red cell folate, the measurement of which can provide additional information.

Other causes of megaloblastic anemia include the effects of chemotherapy, myelodysplastic syndrome or incipient leukemia.

Principle of the Test

In competitive protein binding, the binder should have an equal affinity for the standard and the substance which is present in the patient's serum or plasma. The unlabeled vitamin B$_{12}$ or
Folate competes with its labeled species for the limited number of available binding sites on its specific binder, thus reducing the amount of labeled vitamin B₁₂ or folate bound. Therefore, the level of radioactivity bound is inversely related to the concentration in the patient sample or standard.

In the ICN Pharmaceuticals SimulTRAC* Radioassay Kit, levels of vitamin B₁₂ and folate are determined simultaneously in a single tube. The vitamin B₁₂ and folate tracers, binders and standards are supplied in combined form. The pteroylglutamic acid form of folate (PGA) is used as both standard and tracer in an incubation mixture at pH 9.3. At this pH, both 5-methyltetrahydrofolic acid (MTFA) in the patient specimen and PGA have equal affinity for the milk binder¹¹. The two tracers, [⁵⁷Co] for vitamin B₁₂ and [¹²⁵I] for folate, produce energies at levels which can be easily separated by many commercial two-channel counters.

Analogues of vitamin B₁₂ which bind readily to R protein (non-intrinsic factor) but have negligible affinity for intrinsic factor are in the tracer reagent. These analogues inactivate R protein by saturating its binding sites. Therefore, only intrinsic factor is active as a binder in the assay and only "true" cobalamin is measured.

These reagents may also be used to measure red cell folate to confirm folate deficiency.

**Reagents**

For in vitro diagnostic use

1. **Dithiothreitol Solution**, Catalog No. 06B229237. Contains Dithiothreitol (5%) and stabilizer. (2.0 mL/vial). **Storage**: Refrigerate at 2-8°C; keep tightly closed. **Stability**: Refer to expiration date on vial.

2. **Vitamin B₁₂/Folate** Tracer, Catalog No. 06B262200. Each bottle contains <1.5 µCi (55.5 kBq) [⁵⁷Co] vitamin B₁₂ and <3 µCi (111 kBq) [¹²⁵I] folate in borate buffer with human serum albumin*, dextran, potassium cyanide, non-intrinsic factor blocking agent, dye and preservative (>100 mL/bottle). **Storage**: Refrigerate at 2-8°C; protect from strong light. **Stability**: Refer to expiration date on bottle.

   A. **Working Tracer/DTT Solution Preparation**: Add the contents of one vial of Dithiothreitol Solution to a bottle of Vitamin B₁₂/Folate Tracer. This volume is sufficient for 100 tubes. If less than one bottle of tracer is necessary, dilute one part of Dithiothreitol Solution to each 50 parts of tracer. See assay procedure for an easy-to-use formula for preparing working tracer. **Storage**: Refrigerate at 2-8°C; protect from strong light. **Stability**: One week.

3. **Vitamin B₁₂/Folate Standards A-F**, ready to use; formulated in a synthetic matrix with sodium borate, sodium chloride, and preservatives. **Storage**: Refrigerate at 2-8°C. Protect from strong light. **Stability**: Refer to expiration date on vial.
Concentration

<table>
<thead>
<tr>
<th>Standard</th>
<th>Catalog No.</th>
<th>Volume</th>
<th>pg/mL</th>
<th>pmol/L</th>
<th>ng/mL</th>
<th>nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>06B255820</td>
<td>8 mL</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>06B255821</td>
<td>4 mL</td>
<td>100</td>
<td>74</td>
<td>1.0</td>
<td>2.3</td>
</tr>
<tr>
<td>C</td>
<td>06B255822</td>
<td>4 mL</td>
<td>200</td>
<td>148</td>
<td>2.0</td>
<td>4.5</td>
</tr>
<tr>
<td>D</td>
<td>06B255823</td>
<td>4 mL</td>
<td>400</td>
<td>296</td>
<td>4.0</td>
<td>9.1</td>
</tr>
<tr>
<td>E</td>
<td>06B255824</td>
<td>4 mL</td>
<td>1000</td>
<td>740</td>
<td>10.0</td>
<td>23</td>
</tr>
<tr>
<td>F</td>
<td>06B255825</td>
<td>4 mL</td>
<td>2000</td>
<td>1480</td>
<td>20.0</td>
<td>45</td>
</tr>
</tbody>
</table>

4. **Binder Diluent**, Catalog No. 06B219932, contains sodium borate, sodium chloride, dye and preservatives (11 mL/vial). **Storage**: Refrigerate at 2-8°C. **Stability**: Refer to expiration date on vial.

5. **Vitamin B\textsubscript{12}/Folate Binder**, Catalog No. 06B240214. Lyophilized preparation. Contains folate binder from bovine milk and porcine intrinsic factor, human serum albumin*, dextran and preservatives. **This binder contains R protein; when used with Tracer containing non-intrinsic factor blocking agent, none of the B\textsubscript{12} binding activity is contributed by R protein.** **Preparation**: Add the contents of one vial of Binder Diluent to a vial of binder. Mix gently. **Storage**: Refrigerate at 2-8°C. **Stability**: 8 weeks after reconstitution.

6. **Dextran Charcoal Suspension**, Catalog No. 06B207829, contains charcoal, dextran, sodium chloride, sodium borate and a pelleting aid (100 mL/bottle). **Storage**: Room temperature (18-30°C). **Stability**: Refer to expiration date on bottle.

*CAUTION*: Handle as if capable of transmitting infection: Source material from which this product was derived was found nonreactive for HBsAg and negative for HIV antibody when tested with licensed reagents. No known test method can offer assurance that product derived from human blood will not be infectious. Refer to CDC/NIH Biosafety in Microbiological and Biomedical Laboratories publication (HHS Publication No. [CDC] 84-8395).

**WARNING: CONTAINS RADIOACTIVE MATERIAL**

The ICN Pharmaceuticals 200 tube Radioassay Kit contains <3 microcuries (111 kilobecquerels) of $^{57}$Co and <6 micro-curies (222 kilobecquerels) of $^{125}$I. The ICN Pharmaceuticals 100 tube Radioassay Kit contains <1.5 microcuries (55.5 kilobecquerels) of $^{57}$Co and <3 microcuries (111 kilobecquerels) of $^{125}$I.

This radioactive material may be received, acquired, possessed and used only by physicians, clinical laboratories or hospitals and only for in vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

ICN Pharmaceuticals, Inc.

- Do not eat, drink, smoke or apply cosmetics where radioactive materials are used.
- Do not pipet radioactive solutions by mouth.
- Avoid direct contact with all radioactive materials by using protective articles such as lab coats and disposable gloves.
- All radiological work should be done in a designated area away from traffic.
- Radioactive materials should be stored in their original containers in a designated area.
- A record book for logging receipt and disposal of all radioactive materials should be kept.
- Laboratory equipment and glassware which are subject to contamination should be segregated to prevent cross-contamination of different radioisotopes.
- Any radioactive spills should be taken care of immediately in accordance with established procedures.
- All radioactive materials must be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory.
- Uncontaminated containers may be discarded in non-radioactive waste providing that labels and labeling are defaced.

**Equipment and Supplies Required but Not Provided:**

1. Evacuated glass tube (containing EDTA if desired).
2. Suitable graph paper such as linear, semilog or logit-log paper.
3. Polypropylene tubes (12 x 75 mm), disposable.
4. Test tube rack.
5. Any type semiautomatic pipette with disposable tips capable of delivering 100 µL, 200 µL and 1.0 mL.
6. Glycerin or water bath capable of maintaining 100 ± 2°C.
7. Syringe with Tip, 2 mL, and Metal Holder.
8. Centrifuge capable of achieving an RCF of at least 1000 x g.
9. Gamma counter for measuring \(^{125}\text{I}\) and \(^{57}\text{Co}\) simultaneously or sequentially with adjustable counter settings capable of separating the two counting spectra.

**Equipment and Reagent for Red Cell Folate Assay**

1. Ascorbic acid, ICN Pharmaceuticals, Catalog No. 06B259110.
4. Holder for sealing and carrying microcapillary tubes.
Specimen Collection

Samples should be collected from fasting individuals, since recent food intake may increase the folic acid level appreciably. The laboratory should be advised of possible radioactivity in the sample.

**Samples must not be collected in ascorbic acid or in high concentrations of fluoride, since either of these two agents appears to destroy vitamin B\textsubscript{12}.**

Do not use hemolyzed specimens for serum or plasma folate or vitamin B\textsubscript{12}.

1. **Preparation of Specimen for Analysis:**

   A. **Serum or Plasma:** Collect the blood in a 5 or 10 mL evacuated glass tube. If serum is being collected, allow the blood to clot at room temperature. If plasma is desired use EDTA or heparin as the anticoagulant. Centrifuge for 10 minutes and collect the serum or plasma.

   B. **Whole Blood Hemolysate (for red cell folate assay):** Collect the blood in 5 mL evacuated glass tube containing EDTA.

      Determine and record the hematocrit value.

      Add 100 µL well-suspended blood to 2 mL of freshly prepared 0.2% ascorbic acid solution (w/v). This is a 1:21 dilution. Mix by inversion several times; avoid foaming.

      Let the hemolysate stand at 20 to 25°C for 90 minutes for the complete hydrolysis of polyglutamates by endogenous conjugase. Protect from light during this time.

2. **Shipping of Specimens:** Carefully packed serum, plasma or whole blood hemolysate must be shipped and received frozen.

3. **Storage of Samples:** Store samples before analysis at 2-8°C. If storage is expected to exceed 4 hours, the sample should be stored at -20°C or below. Samples are stable for 6-8 weeks at this temperature. Do not store in freezers with an automatic defrost cycle in order to avoid repeated freezing and thawing.

**Procedure**

If the counter has two or more channels it should be calibrated to count \([^{125}\text{I}]\) in one channel and \([^{57}\text{Co}]\) in another channel.

If the counter has one channel, it will be necessary to count the tubes twice, once for each isotope.

All reagents and samples must be brought to room temperature before use and returned to recommended storage temperature immediately after use. Do not use reagents other than those provided in this kit.
CAUTION: Vitamin B₁₂ and folates are light sensitive and should be exposed to diffuse light only, for as short a period of time as possible. It is advisable to cover the rack of assay tubes during the extraction and binding steps.

The following protocol recommends that the standards be run in duplicate. Patient samples must be assayed in duplicate and the preparation of the standard curve and the clinical determinations must be run simultaneously. Control sera should be run at the same time as patient samples.

Reagent Preparation

1. If the entire contents of one bottle of tracer cannot be used within one week, use the following equation to calculate the amounts of DTT to be added to the Tracer/Buffer:

\[
\frac{\text{mL Tracer needed} \times 2}{100} = \text{mL of DTT}
\]

For example,

\[
\frac{35 \text{ mL of tracer needed} \times 2}{100} = 0.7
\]

Therefore 0.7 mL of DTT must be added to 35 mL of Tracer/Buffer prior to starting the assay.

2. Add the contents of one vial of Binder Diluent to one vial of Binder. Mix gently to dissolve. This reagent is stable for 8 weeks after reconstitution.

Assay Procedure

1. Number 16 polypropylene tubes for the standards. Beginning with 17, number two tubes for each clinical sample.

2. Add standards and clinical samples as follows according to the outline

3. Add 1000 µL Working Tracer/Buffer Solution to all tubes including the Total Count tubes (1 and 2).

4. Mix gently and cover all tubes (except 1 and 2) with aluminum foil.

5. Heat all tubes (except 1 and 2) in a glycerine or water bath at 100°C for 15 minutes; do not begin timing until the bath returns to a rolling boil or 100°C. Protect from light.

6. Remove all tubes from 100°C bath. Cool to (20-25°C) in a running water bath. Do not continue assay until the tubes are within this range. Uncover all tubes.
7. Add 100 µL Binder to tubes 5-16 and sample tubes. Mix gently by hand.

8. Incubate tubes 3-16 and all sample tubes at room temperature (18-25°C) for 30 minutes from the time of the last addition of the binder. Cover the rack of tubes with aluminum foil to exclude light, or keep in a dark location.

9. Add 0.4 mL DCC suspension to tubes 3-16 and to all sample tubes (17,18, etc.). Do not add to tubes 1 and 2. This reagent is "squirited" into each tube to obtain a uniform suspension in the reaction mixture. A syringe and metal holder should be used. Avoid excessive foaming of the mixture by squirting at a slight angle down the wall of the tube.

10. Keep all tubes at room temperature (18 - 25°C) for 10 minutes after completion of step 9. Contact time with the charcoal must be as close to identical as possible for standards and patient samples.

11. Centrifuge at a minimum of 1000 x g for 10 minutes, preferably in the cold.

12. Consecutively number a set of clean polypropylene or polystyrene tubes, beginning with 3.

13. Gently decant each clear supernatant into the similarly numbered tube prepared in step 12. Maximal transfer is obtained by hitting the rims together. Avoid decanting over any charcoal to the counting tube. Discard the charcoal residues. (See note below.)

14. Count the radioactivity in the supernatants and in tubes 1 and 2 in sequence for one minute with a gamma counter. The total count per minute for tubes 1 and 2 for $^{57}$Co should be between 10,000 and 20,000 and for $^{125}$I between 15,000 and 35,000, depending on the instrument and age of the tracer. A shorter counting time may be used provided the counts in tubes 1 and 2 are at least 10,000 (total count).

**Note:** We recommend counting bound activity. However, should you wish to count the free, decant the supernatant into radioactive waste and count the residue. Use the counts from tubes 3 and 4 as total counts for calculation. Do not count tubes 1 and 2.
VITAMIN B\textsubscript{12}/FOLATE RADIOASSAY

<table>
<thead>
<tr>
<th>Tube</th>
<th>Standard (µL)</th>
<th>Patient Serum of Plasma (µL)</th>
<th>Working Tracer Solution (µL)</th>
<th>Incubate</th>
<th>Binder (µL)</th>
<th>Incubate</th>
<th>Charcoal Suspension (µL)</th>
<th>Incubate</th>
<th>Centrifuge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2</td>
<td>---</td>
<td>---</td>
<td>1000</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.4 mL</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>3, 4</td>
<td>200 A</td>
<td>---</td>
<td>1000</td>
<td>Incubate all tubes at 100°C for 15 min.</td>
<td>100</td>
<td>Incubate all tubes at room temp. for (18-25°C) 30 min.</td>
<td>0.4 mL</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>5, 6</td>
<td>200 A</td>
<td>---</td>
<td>1000</td>
<td>---</td>
<td>100</td>
<td>---</td>
<td>0.4 mL</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>7, 8</td>
<td>200 B</td>
<td>---</td>
<td>1000</td>
<td>---</td>
<td>100</td>
<td>---</td>
<td>0.4 mL</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>9, 10</td>
<td>200 C</td>
<td>---</td>
<td>1000</td>
<td>---</td>
<td>100</td>
<td>---</td>
<td>0.4 mL</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>11, 12</td>
<td>200 D</td>
<td>---</td>
<td>1000</td>
<td>---</td>
<td>100</td>
<td>---</td>
<td>0.4 mL</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>13, 14</td>
<td>200 E</td>
<td>---</td>
<td>1000</td>
<td>---</td>
<td>100</td>
<td>---</td>
<td>0.4 mL</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>15, 16</td>
<td>200 F</td>
<td>---</td>
<td>1000</td>
<td>---</td>
<td>100</td>
<td>---</td>
<td>0.4 mL</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Samples</td>
<td>---</td>
<td>200</td>
<td>1000</td>
<td>---</td>
<td>100</td>
<td>---</td>
<td>0.4 mL</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

- After centrifugation, decant the supernatants into the clean tubes and count the radioactivity.
- Calculate.
- Draw standard curve and determine patient assay values.

Calculation of Result

A. **Serum or Plasma Vitamin B\textsubscript{12} and Folate**: The vitamin B\textsubscript{12} curve and values are calculated from the data obtained by counting $[^{57}\text{Co}]$; the folate curve and values are calculated from the $[^{125}\text{I}]$ counts.

1. Average the counts found in tubes 3 and 4, the "Blank" tubes. Subtract the "Blank" from all other tube counts to obtain the corrected counts. Use only the corrected counts in the calculations. **Note: The unit of time must be constant for all tubes counted.**

2. Average the corrected counts for tubes 1 and 2 to give the corrected Total Count per assay.

3. Divide the average of the corrected counts for tubes 5 and 6 by the corrected Total Count to give the Trace Binding, $B_0$. This value should be greater than 40%.

4. Divide the corrected counts for each tube by the average corrected counts for tubes 5 and 6 to give the % of Trace Binding for each tube.

5. A Standard Curve may be plotted as follows:

Using logit-log paper, plot % of Trace Binding as the ordinate versus pg/mL Vitamin B\textsubscript{12} or ng/mL Folate Standard on the log scale. Typical counting data and calculated % of Trace Binding are given in Table 1, the standard curves plotted for these data are shown in Figure 1. In practice, it may be advisable to plot each standard curve on a separate sheet to avoid possible confusion.
6. The concentration of Vitamin B$_{12}$ or folate in serum or plasma is determined by interpolation from the standard curve of % of Trace Binding versus either pg/mL vitamin B$_{12}$ or ng/mL folate (Figure 1).

7. Example: The following example is for a folate sample. The same calculation procedure is used for vitamin B$_{12}$.

$$\text{Blank} = \frac{\text{counts tube 3} + \text{counts tube 4}}{2} = \frac{1072 + 953}{2} = 1012$$

$$\text{Corrected Total Count} = \frac{(\text{counts tube 1-Blank})+ (\text{counts tube 2-Blank})}{2}$$

$$= \frac{(32213 - 1012) + (32864 - 1012)}{2} = 31526$$

$$\text{B}_o = \text{Trace Binding} = \frac{\text{average corrected counts for tubes 5 and 6} \times 100}{\text{corrected Total Count}}$$

$$= \frac{19768 \times 100}{31526} = 62.7\%$$

$$\% \text{ of Trace Binding calculation for Tube 7} = \frac{(\text{counts tube 7 - Blank})}{\text{average corrected counts for tubes 5 and 6}} \times 100$$

$$= \frac{(13649 - 1012)}{19768} \times 100 = 63.9\%$$

Sample Calculation for a Patient Specimen:

$$\text{Count (found)} = 6455$$

$$\text{Blank} = 1012$$

$$\% \text{ of Trace Binding} = \frac{6455 - 1012}{19768} \times 100 = 27.5\%$$

The logit-log Standard Curve (Figure 1) shows that 27.5% corresponds to a folate concentration of 4.2 ng/mL.

The average of this value and the value for the duplicate determination is reported as the folate concentration in ng/mL for the patient sample.

8. To calculate results using charcoal residues, plot counts obtained for standards vs. concentration on semi-log paper, or for a linear plot, plot a standard curve on the logit-log paper provided as described in Step 5 above.
% of Trace Binding = $A - B \times 100$
\[\frac{A - C}{A} \times 100\]

Where
- $A = \text{average residue count, tubes 3 and 4}$
- $B = \text{sample residue count}$
- $C = \text{average residue count, tubes 5 and 6}$

B. **Red Cell Folate:**

1. Calculate the % of Trace Binding for the hemolysate (Step A-4).

2. Obtain the concentration of folate by interpolation from the Standard Curve (Step A-5).

3. Multiply the folate concentration by 21. [A 1:21 dilution of whole blood was made in preparing the specimen.] This gives the folate concentration in ng/mL of whole blood.

4. Divide the folate concentration of whole blood by the hematocrit expressed as a decimal. This gives the folate concentration in ng/mL of packed red cells.

\[
\text{ng/mL of packed red cells} = \frac{(\text{ng/mL of hemolysate}) \times 21}{\% \text{ hematocrit/100}}
\]

**Sample Calculation for Red Cell Folate:**

- Count (found) = 6112
- Blank = 1012
- % of Trace Binding = 25.8%

The logit-log Standard Curve (Figure 1) shows that 25.8% corresponds to a folate concentration of 4.8 ng/mL.

\[
\text{Patient hematocrit} = 42\%
\]

\[
\text{ng/mL of packed red cells} = \frac{4.8 \times 21}{0.42} = 240 \text{ ng/mL}
\]

The average of this value and the value for the duplicate determination is reported as the red cell folate concentration in ng/mL for the patient sample.

**NOTE:** It is not routinely necessary to correct for serum/plasma folate since this value is very small compared to that of red cell folate. Occasionally elevated serum or plasma folate levels will occur. If the serum or plasma folate concentration is greater than 10% of the calculated red cell folate concentration, a serum correction should be made as follows:

\[
\text{ng/mL of packed red cells} = (\text{ng/mL} \times 21) - [\text{serum folate} (1\%-\text{hematocrit/100})]
\]
% hematocrit/100
Sample Calculation:

1. Serum or plasma folate concentration = 32 ng/mL
2. Folate concentration of hemolysate = 3.0 ng/mL
3. Hematocrit for this patient = 30 %
4. Uncorrected red cell folate concentration:
   \[
   \frac{3.0 \text{ ng/mL} \times 21}{0.30} = 210 \text{ ng/mL}
   \]

Since 32 ng/mL is greater than 10% of 210 ng/mL, the red cell folate concentration must be corrected as follows:

\[
\text{corrected ng/mL of packed red cells} = \frac{(3.0 \text{ ng/mL} \times 21) - [32 \text{ ng/mL} \times (1-0.3)]}{0.30}
\]

Corrected red cell folate = 135 ng/mL

Limitations of the Procedure

1. Radioassay methodologies for determining vitamin B\textsubscript{12} content of patient specimens may provide values for populations at risk different from the values obtained using specific microbiological methodologies, since each has its own reference range.
2. Accurate usage of the SimulTRAC\textsuperscript{*} Kit depends on the ability of the gamma counter to discriminate between disintegrations of \textsuperscript{125}I and \textsuperscript{57}Co. Windows must be set so that there is minimal interference from \textsuperscript{57}Co in the \textsuperscript{125}I channel and vice-versa. This method may be used to determine spillover.
   a. Set the gamma counter to count \textsuperscript{125}I.
      1. Count \textsuperscript{125}I source. = A
      2. Count \textsuperscript{57}Co source in \textsuperscript{125}I setting. = B
   b. Set the gamma counter to count \textsuperscript{57}Co.
      1. Count \textsuperscript{57}Co source. = C
      2. Count \textsuperscript{125}I source in \textsuperscript{57}Co setting. = D
   c. Calculate spillover
      1. of \textsuperscript{57}Co into \textsuperscript{125}I = \frac{B \times 100}{C}
      2. of \textsuperscript{125}I into \textsuperscript{57}Co = \frac{D \times 100}{A}
Spillover should not exceed 3% for either channel. If more than 3% spillover is detected, the window setting must be narrowed or a mathematical method of correction applied. [⁵⁷Co] sources are available upon request.

3. Clinical samples containing radioactivity from prior treatment or studies may lead to erroneous results.

4. **Caution should be used in selecting commercial folate control sera.** Some controls may have been prepared with unstable 5-methyltetra-hydrofolate (MTFA), leading to unexpectedly low values. Other sera prepared with pteroylglutamic acid but analyzed at pH 7.4 using MTFA standards will be assigned too high a folate value by the manufacturer. These control sera will give a folate value lower than that indicated by the package insert.

5. Expected values and control ranges determined by one method of calculation may not be identical to those determined by an alternate method.

**Expected Values**

Each laboratory must define its own characteristics for interpretation of results. The expected ranges shown should serve only as a guide.

The normal range for serum folate was determined from the values obtained from the central 95% of a population of 117 volunteers judged to be hematologically normal by standard laboratory criteria. The range obtained was 1.6-13.2 ng/mL. Due to the significant fluctuation of serum folate values with a recent change in the patient's diet, an indeterminate range is recommended. The lowest value of the central 90% of the normal population was established as the high end of the indeterminate range. The lowest value of the central 95% of the normal population was established as the high end of the folate deficient range.

The expected range for red cell folate is based on the log normal distribution as measured in over 120 healthy volunteers. The expected range calculated as ± 2 S.D. from the log mean of the distribution was found to be 125-600 ng/mL.

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>Folate (ng/mL)</th>
<th>Folate (nmol/L)</th>
<th>Red Cell Folate (ng/mL)</th>
<th>Red Cell Folate (nmol/L)</th>
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<tr>
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<td>&lt; 284</td>
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<td>&gt; 2.2</td>
<td>&gt; 5.0</td>
<td>&gt; 125</td>
<td>&gt; 284</td>
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</tbody>
</table>

For the Vitamin B₁₂ expected values, two populations were used. The first population consisted of 70 subjects with confirmed diagnoses of pernicious anemia. The estimated 99.5 percentile of this population defined the upper limit of the deficient (low) range. The second population consisted of 157 healthy male and female volunteers ranging in age from 19 to 60 with no unusual dietary habits. These volunteers were judged to be hematologically normal by standard laboratory criteria. The central 95 percent of this population defined the expected...
range (normal). The indeterminate range is the range of values between deficient and normal populations.

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<tr>
<th>Interpretation</th>
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<tr>
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<td>pg/mL</td>
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<tr>
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<tr>
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<td>210 - 920</td>
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<td>&gt; 920</td>
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A graphic comparison of the results obtained for these two populations by the Vitamin B\(_{12}\) assay and the Euglena gracilis microbiological assay is shown in Figure 2.

Serum vitamin B\(_{12}\) levels well above 1000 pg/mL are suggestive of either liver disease or a myeloproliferative disorder such as polycythemia vera, myeloid metaplasia or chronic granulocytic leukemia. Levels above 4000 pg/mL are unusual in liver disease, but are common in myeloproliferative disorders\(^8,9,12,13\).

**WHENEVER LABORATORY DATA CONFLICT WITH CLINICAL FINDINGS OR IMPRESSIONS, CLINICAL JUDGEMENT SHOULD BE EXERCISED AND ADDITIONAL EVALUATION UNDERTAKEN.**

**Specific Performance Characteristics**

**Accuracy:**

Comparison of Vitamin B\(_{12}\) results with those obtained by microbiological assay with Euglena gracilis\(^14\) gave the following calculated regression data (x = microbiological assay, y = SimulTRAC* vitamin B\(_{12}\)):

- Number of samples = 227
- Correlation coefficient = 0.954
- Slope = 1.44
- Intercept = -5.0

A comparison of the serum folate values obtained with this kit (y), with those obtained using the previous SimulTRAC* Slurry kit (x) gave these regression data:

- Number of samples = 20
- Correlation coefficient = 0.99
- Slope = 1.05
- y Intercept = -0.013
**Precision:**

1. **Intra-assay variation:**

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<tr>
<th>Sample</th>
<th>Vitamin B&lt;sub&gt;12&lt;/sub&gt;</th>
<th>Folate</th>
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2. **Inter-assay variation:**

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**Specificity:**

5-Methyltetrahydrofolic acid and pteroylglutamic acid have equal affinity for the binder in this assay<sup>11</sup>.

Cobinamide (a vitamin B<sub>12</sub> analogue) was less than 0.02% cross-reactive.

**Sensitivity:**

As defined by the concentration at 90% trace binding, sensitivity is 60 pg/mL for Vitamin B<sub>12</sub> and 0.25 ng/mL for folate.
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<th>Average cpm</th>
<th>% Bound</th>
<th>% Trace Binding</th>
<th>Vitamin B&lt;sub&gt;12&lt;/sub&gt; pg/mL</th>
<th>Vitmain B&lt;sub&gt;12&lt;/sub&gt; pmol/L</th>
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Figure 1
SimulTRAC* Vitamin B₁₂/Folate Radioassay
Typical Standard Curves with Logit-Log Transformation
Figure 2

Hematologic ≠ True Cobalamin vs Euglena gracilis

- Normal (Normal Blood Morphology) n = 160
- Low (Visually Depleted) n = 70

Vitamin B₁₂ pg/mL
References
