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06B58581-R13
QER Q00-182, EOF-419, 420
8/00

SimulTRAC-SNB

Catalog No.: 06B257117 - 100 Tube Kit
06B264806 - 200 Tube Kit

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SimulTRAC-SNB
RADIOASSAY KIT VITAMIN B_{12} [^{57}Co]/FOLATE [^{125}I]

For the Simultaneous Quantitative Determination of Vitamin B_{12} and Folate in Serum and Plasma.

I. SUMMARY AND EXPLANATION OF THE TEST:

Vitamin B_{12} and folate deficiencies are two of the three unequivocal nutritional anemias of man, the third being that due to lack of iron[1].

Folate (folic acid) deficiency is present in about one-third of all pregnant women, the vast majority of alcoholics, the majority of people who eat a diet devoid of raw fruits and vegetables or fresh fruit juices, many people with structural or functional damage in the upper third of the small bowel and in a number of other situations[2]. Measurement of folate levels constitutes a direct and reliable means of determining the existence of folate deficiency[3], and this test should be performed for every patient who has a megaloblastic anemia, as well as every patient who has anemia, hypersegmentation of the granulocytic nuclei, and coincident evidence of iron deficiency[4].

Vitamin B_{12} is essential for normal folic acid metabolism. It is advisable to determine serum vitamin B_{12} and red cell folate in addition to serum folate to ascertain that the diagnosis is folate deficiency, for which the proper treatment is folic acid. A low red cell folate can also mean that the patient has primary vitamin B_{12} deficiency, blocking the ability of cells to take up folate, in which case the proper therapy would be vitamin B_{12} rather than folic acid[5].

Vitamin B_{12} deficiency is most frequently associated with pernicious anemia, gastric damage, intestinal damage and pure vegetarianism. The only significant dietary sources of vitamin B_{12} are of animal origin; therefore, a purely vegetarian diet will eventually lead to vitamin B_{12} deficiency.

Morphologic alterations of the blood cells associated with vitamin B_{12} deficiency are also produced by folic acid deficiency, and determination of serum cobalamin level is necessary to determine whether the megaloblastosis is due to deficiency of B_{12} or folate or both[2,4,6-8].

II. 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 sample. 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 B_{12} 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-SNB Radioassay Kit, levels of vitamin B_{12} and folate are determined simultaneously in a single tube. The vitamin B_{12} 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 9.5. At this binding pH, both 5-methyltetrahydrofolic acid (MTFA) in the patient sample and PGA in the standards have equal affinity for the milk binder. The two tracers, [^{57}Co] for vitamin B_{12} and [^{125}I] for folate, produce energies at levels which can be easily separated by many commercial two-channel counters.

The ICN Pharmaceuticals SimulTRAC-SNB kit uses purified intrinsic factor. The R protein, which has high affinity for cobalamin (vitamin B_{12}) analogues in human plasma, has been physically removed by affinity chromatography. With the R protein removed, only purified intrinsic factor is available for binding; because it is specific for cobalamin, a "true" cobalamin value is measured[9,10]. Both purified intrinsic factor and folate binder have been covalently linked to a solid support.

In this ICN Pharmaceuticals procedure, endogenous serum binders for both vitamin B_{12} and folate are destroyed after incubation with tracer/ dithiothreitol for 15 minutes followed by a 10 minute extraction reaction at alkaline pH (12-13). This eliminates the need for heating the sample at 100°C. To confirm folate deficiency, this kit may also be used to measure red cell folate.

III. REAGENTS:

For *in vitro* diagnostic use

A. **SimulTRAC-SNB Dithiothreitol Solution,** Catalog No. 06B229253. Contains Dithiothreitol in phosphate buffer with stabilizer. >10 mL/vial. One vial/100 tube kit, 2 vials/200 tube kit. **Storage:** Refrigerate at 2-8°C; keep tightly closed. **Stability:** Refer to expiration date on vial.

B. **SimulTRAC-SNB Vitamin B_{12}/ Folate Tracer,** Catalog No. 06B257133. A bottle contains <1.5 μCi (55.5 kBq) [^{57}Co] Vitamin B_{12} and <3 μCi (111 kBq) [^{125}I] folate in borate buffer with human serum albumin*, dextran, potassium cyanide, endogenous binder blocker, dye and preservative. Volume: >10 mL/bottle. One bottle/100 tube kit, 2 bottles/200 tube kit. **Storage:** Refrigerate at 2-8°C; protect from strong light. **Stability:** Refer to expiration date on bottle.

1. **Working Tracer/DTT Solution,** Preparation: Add the contents of one vial of Dithiothreitol Solution to one bottle of Vitamin B_{12}/Folate Tracer. This volume is sufficient for 100 tubes. If the entire contents of one bottle of tracer cannot be used within 30 days after DTT addition, dilute one part of Dithiothreitol Solution with one part of tracer. **Storage:** Refrigerate at 2-8°C; protect from strong light. **Stability:** Thirty days after preparation

C. **SimulTRAC-SNB Binder,** Catalog No. 06B257150. Contains purified folate binder from bovine milk and purified porcine intrinsic factor bound to a solid support in borate buffer with sodium chloride, dye and preservative. >100 mL/bottle. One bottle/ 100 tube kit, 2 bottles/200 tube kit. ***Shake vigorously before use.*** **Storage:** Refrigerate at 2-8°C. **Stability:** Refer to expiration date on bottle.

D. **SimulTRAC-SNB Blank Reagent,** Catalog No. 06B254975, contains solid support without binder, formulated at the same solid phase concentration as the binder, in borate buffer with sodium chloride, dye and preservative. Volume: >8 mL/bottle. One bottle/100 tube kit, two bottles/200 tube kit. **Storage:** Refrigerate at 2-8°C. **Stability:** Refer to expiration date on bottle.

E. **SimulTRAC-SNB Vitamin B_{12}/ Folate Standards A-F,** Vitamin B_{12} (cyanocobalamin) and Folic Acid (PGA) in borate buffer with human serum albumin*, sodium chloride, stabilizer and preservatives. One vial each standard/kit. **Storage:** Refrigerate at 2-8°C; protect from strong light. **Stability:** Refer to expiration dates on vials.

			Concentration - Vitamin B_{12}		Concentration - Folic Acid	
Standard	Cat. No.	Volume	pg/mL	pmol/L	ng/mL	nmol/L
A	06B254851	6 mL	0	0	0	0
B	06B254860	3 mL	100	74	1.0	2.3
C	06B254878	3 mL	200	148	2.0	4.5
D	06B254886	3 mL	400	296	4.0	9.1
E	06B254916	3 mL	1000	740	10.0	23
F	06B254924	3 mL	2000	1480	20.0	45

F. **Extracting Reagent,** Catalog No. 06B257176, contains 1.0N sodium hydroxide with organic extracting enhancer and yellow dye. Volume: >10 mL/bottle. One bottle /100 tube kit, 2 bottles/200 tube kit. **Storage:** Refrigerate at 2-8°C. **Stability:** Refer to expiration date on bottle.

CAUTION: Avoid contact with eyes, skin and clothing. Take appropriate safety precautions when pipetting this reagent.

***WARNING:** 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 products 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

This ICN Pharmaceuticals Radioassay Kit contains <3 microcuries (111 kilobecquerels) of [^{125}I] and <1.5 microcuries (55.5 kilobecquerels) of [^{57}Co] per vial of tracer. 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.

Adherence to the basic rules of radiation safety should provide adequate protection. The user is referred to National Bureau of Standards Handbook No. 92, "Safe Handling of Radioactive Materials", issued March 9, 1964, Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402. A summary follows:

♦ 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.

G. Equipment and Reagents Required but Not Provided in Kit:

Evacuated glass tube, 5 or 10 mL or Evacuated glass tube containing EDTA, 7 or 10 mL.

Polypropylene or polystyrene tubes (12 x 75 mm), disposable.

Test tube rack.

Any type semi-automatic pipette with disposable tips capable of delivering 100 µL, 200 µL and 1.0 mL.

Vortex mixer.

Centrifuge capable of achieving an RCF of at least 1000 x g.

Gamma counter for measuring [^{125}I] and [^{57}Co] simultaneously or sequentially with adjustable counter settings capable of separating the two counting spectra.

H. Additional Equipment and Reagents Required for Red Cell Folate Assay:

Ascorbic acid, ICN Pharmaceuticals, Catalog No. 06B259110.

Micro-Hematocrit Centrifuge.

Micro-Hematocrit Capillary Tubes/Heparinized.

Holder for sealing and carrying microcapillary tubes.

IV. 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_{12}.

Do not use hemolyzed specimens for serum or plasma assays.

A. Preparation of specimen for analysis:

1. 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 in the closed tube for 30-60 minutes. Use EDTA if plasma is desired for analysis. Centrifuge for 10 minutes and collect the serum or plasma.

2. Whole Blood Hemolysate (for red cell folate assay)

Collect the blood in a 7 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 60 to 90 minutes prior to assay. Protect from light during this time.

B. Shipping of Specimens:

Carefully packed serum and plasma should be shipped and received frozen.

C. 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.

V. PROCEDURE:

If the counter has two or more channels it should be calibrated to count [^{125}I] in one channel and [^{57}Co] in another channel. If the counter has one channel it should be calibrated so that different counter settings will count one isotope at a time. In the case of the latter, it will be necessary to count the tubes twice, once setting the counter for [^{125}I] and obtaining the folate curve and sample data, then setting the counter for [^{57}Co] to obtain the vitamin B_{12} curve and sample data. All reagents and samples must be brought to room temperature before use. Return to recommended storage temperature after use. Do not use reagents other than those provided in this particular kit. Vitamin B_{12} 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.

In the following protocol it is recommended that the standard level points 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.

A. Reagent Preparation:

1. If one bottle of tracer can be used within 30 days, add the contents of one vial of DTT to a bottle of tracer. If the same bottle of tracer is to be used for periods longer than 30 days, follow the directions below.

2. Equal amounts (100 µL each) of tracer and dithiothreitol (DTT) are added per assay tube. Mix tracer and dithiothreitol 1 to 1 and use 200 µL/tube. Alternatively the tracer and DTT may be pipetted separately, 100 µL/tube of each, pipetting the tracer first followed by the dithiothreitol.

B. Assay Procedure:

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

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

3. Add 200 µL Working Tracer/ DTT Solution (Reagent 2A) to all tubes including the Total Count tubes (1 and 2). Vortex.

4. Incubate for 15 minutes at room temperature.

5. Add 100 µL Extracting Reagent to tubes 3-16 and all sample tubes. Vortex.

6. Incubate for 10 minutes at room temperature.

7. Thoroughly mix the bottle of SimulTRAC-SNB Blank Reagent. Add 1000 µL blank reagent to tubes 3 and 4.

8. Thoroughly mix the bottle of SimulTRAC-SNB Binder. ***Caution: Must be shaken vigorously.*** Add 1000 µL binder to tubes 5-16 and all sample tubes. Vortex.

9. Incubate tubes 3-16 and all sample tubes at room temperature for 60 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.

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

11. Gently decant and discard each supernatant. Remove the last drop by touching the tube to a paper towel or absorbent paper.

12. Count the radioactivity in the pellets 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 25,000 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).

SimulTRAC-SNB RADIOASSAY

Tube	Standard or Sample (µL)	Working Tracer Solution (µL)	Incubate	Extracting Reagent (µL)	Incubate	Blank Reagent (µL)	Binder (µL)	Incubate	Centrifuge
1, 2	---	200	Vortex.	---	Vortex.	---	---	Vortex.	Centrifuge
3, 4	200A	200	Incubate	100	Incubate	1000	---	Incubate	all tubes
5, 6	200A	200	all tubes	100	all tubes	---	1000	all tubes	(except 1
7, 8	200B	200	at room	100	at room	---	1000	at room	and 2) at
9,10	200C	200	temper-	100	temper-	---	1000	temper-	1000 x g
11,12	200D	200	ature for	100	ature for	---	1000	ature for	for 10
13,14	200E	200	15 min.	100	10 min.	---	1000	60 min.	min.
15,16	200F	200		100		---	1000		
Patient Samples	200	200		100		---	1000		

After centrifugation, decant the supernatants and count the radioactivity in the pellets.

Calculate. Draw standard curve and determine patient assay values.

VI. CALCULATION OF RESULTS:

A. Serum or Plasma Vitamin B_{12} and Folate

The Vitamin B_{12} curve and values are calculated from the data obtained by counting [^{57}Co]; the folate curve and values are calculated from the [^{125}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_o. This value should be greater than 35%.

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_{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:

a. The following example is for a B_{12} sample. The same calculation procedure is used for folate:

$$\text{Blank} = \frac{\text{counts tube 3 + counts tube 4}}{2} \quad \frac{= 750 + 728}{2} = 739$$

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

$$= \frac{(23319 - 739) + (23716 - 739)}{2} = 22778$$

$$\text{Bo = Trace Binding} = \frac{\text{av. corr. counts for tubes 5 \& 6}}{\text{corrected Total count}} \times 100 = \frac{10632}{22778} \times 100 = 46.7\%$$

% of Trace Binding

$$\text{calculation for Tube 7} = \frac{\text{(counts tube 7 - Blank)}}{\text{av. corr. counts for tubes 5 \& 6}} \times 100 = \frac{(9914 - 739)}{10632} \times 100 = 86.2\%$$

Sample Calculation for a Patient Specimen:

Count (found) = 7324

Blank = 739

$$\text{\% of Trace Binding} = \frac{7324 - 739}{10632} \times 100 = 61.9\%$$

The logit-log Standard Curve (Figure 1) shows that 61.9% corresponds to a B_{12} concentration of 470 pg/mL.

The average of this value and the value for the duplicate determination is reported as the B_{12} concentration in pg/mL for the patient sample.

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-6).

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.

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

Sample Calculation for Red Cell Folate:

Count (found) = 11271

Blank = 1321

% of Trace Binding = 56.3

The logit-log Standard Curve (Figure 1) shows that 56.3% corresponds to a folate concentration of 4.6 ng/mL.

Patient hematocrit = 42%

$$\text{ng/mL of packed red cells} = \frac{4.6 \times 21}{0.42} = 230 \text{ ng/mL}$$

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:

$$\text{ng/mL of packed red cells} = \frac{\text{(ng/mL x 21) - [serum folate (1 - \%hematocrit/100)]}}{\text{\% hematocrit/100}}$$

C. 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:

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

corrected red cell folate = 135 ng/mL

VII. LIMITATIONS OF THE PROCEDURE:

A. Radioassay methodologies for determining vitamin B_{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.

B. Occasional spuriously elevated serum Vitamin B_{12} results have been reported with several "No Boil" type assays.[11,12] It has been reported that these results

may be due to presence in the sample of anti-intrinsic factor blocking antibodies or endogenous binders to Vitamin B_{12} which may be incompletely inactivated by the alkaline denaturation reagents.[12] Although this type of discrepancy appears to be rare, results obtained with "No Boil" assays should be interpreted with caution; where appropriate the "No Boil" result can be confirmed by an alternate "Boil" method (ICN Catalog No. 06B254819). When results conflict with clinical findings or impressions, clinical judgement should be exercised and additional evaluation undertaken.

C. Accurate usage of the SimulTRAC Kit depends on the ability of the gamma counter to discriminate between disintegrations of [^{125}I] and [^{57}Co]. Windows must be set so that there is minimal interference from [^{57}Co] in the [^{125}I] channel and vice-versa.

D. This method may be used to determine spillover.

1. Set the gamma counter to count [^{125}I].

a. Count [^{125}I] source. =A b. Count [^{57}Co] source in [^{125}I] setting. = B

2. Set the gamma counter to count [^{57}Co].

a. Count [^{57}Co] source. = C b. Count [^{125}I] source in [^{57}Co] setting. = D

3. Calculate spillover

a. of [^{57}Co] into [^{125}I] = $\frac{B}{C}$ x 100 b. of [^{125}I] into [^{57}Co] = $\frac{D}{A}$ x 100

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. [^{57}Co] sources are available upon request.

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

5. Caution should be used in selecting commercial folate control sera. Some controls may have been prepared with unstable 5-methyltetrahydrofolate (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.

6. Expected values and control ranges determined by one method or calculation may not be identical to those determined by an alternate method. Consistent use of one data reduction method is recommended for greater precision.

VIII. EXPECTED VALUES:

Each laboratory must define its own characteristics for interpretation of results, but the expected ranges shown may serve as a guide.

The normal range for serum folate was determined from the values obtained from the central 95% of a normal population (1.5-16.9 ng/mL). The normal population

consisted of 121 specimens freshly drawn from volunteers judged to be hematologically normal by standard laboratory criteria.

The expected range for red cell folate was determined by assaying whole blood hemolysates from 117 subjects found to be hematologically normal. The central 95% of all the normals assayed was found to be 120-860 ng/mL.

	Serum Folate		Red Cell Folate	
Interpretation	ng/mL	(nmol/L)	ng/mL	(nmol/L)
Low	<1.5	<3.4	<120	<272
Normal	>1.5	>3.4	>120	>272

For serum vitamin B_{12} ("true" cobalamin) expected values, two populations were used. The first population consisted of 38 vitamin B_{12} deficient subjects with confirmed diagnoses including pernicious anemia, gastric or intestinal damage, or disease states associated with B_{12} deficiency. The estimated 99 percentile of this population defined the upper limit of the deficient (low) range.

The second population consisted of 121 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 defined as the range of values between deficient and normal populations.

Interpretation	Vitamin B_{12} pg/mL	(pmol/L)
Low	<120	<88
Indeterminate	120 - 160	88 - 118
Normal	160 - 970	118 - 716
High	>970	>716

In another study specimens were compared by the SimulTRAC-SNB assay and the *Euglena gracilis* microbiological assay. This 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[8,9,13,14].

IX. SPECIFIC PERFORMANCE CHARACTERISTICS:

A. Accuracy:

1. A comparison of the results obtained for vitamin B_{12} with this kit and those obtained using the SimulTRAC-S Radioassay kit gave these regression data:

(x = SimulTRAC-S, y = SimulTRAC-SNB)

Number of samples = 160 Correlation coefficient = 0.98

Slope = 0.93 Y-Intercept = -26 pg/mL

2. Comparison of results with those obtained by microbiological assay with *Euglena gracilis*[15], gave the following calculated regression.

(x = microbiological assay, y = SimulTRAC-SNB):

Number of samples =132 Correlation coefficient =0.96

Slope =1.1 Y-Intercept =-32 pg /mL

3. A comparison of the serum folate results obtained in this kit with those obtained using the SimulTRAC-S kit gave the following results:

Number of samples =160 Correlation coefficient =0.97

Slope =1.03 Y-Intercept =-0.01 ng/mL

B. Precision:

1. Intra-assay variation:

a. **Vitamin B_{12}**

Specimen	N	Mean pg/mL	S.D.	% C.V.
Control 1	20	318	19.5	6.1
Control 2	22	444	14.1	3.2
Control 3	20	593	26.3	4.4
Control 4	20	1203	69	5.7
Serum Pool	20	169	19	11.2

b. **Folate**

Specimen	N	Mean ng/mL	S.D.	% C.V.
Control 1	20	1.87	0.16	8.6
Control 2	22	2.16	0.15	6.9
Control 3	20	4.89	0.25	5.1
Control 4	20	12.1	0.54	4.5
Serum Pool	20	6.10	0.25	4.1

2. Inter-assay variation:

a. Vitamin B_{12}

Specimen	N	Mean pg/mL	S.D.	% C.V.
Control 1	35	313	25.6	8.2
Control 2	44	432	27.9	6.4
Control 3	52	555	38	6.8
Control 4	52	1106	47	4.2
Serum Pool	30	151	18.6	12.3

b. **Folate**

Specimen	N	Mean ng/mL	S.D.	% C.V.
Control 1	35	1.62	0.19	11.7
Control 2	44	1.96	0.16	8.2
Control 3	52	4.50	0.34	7.5
Control 4	52	10.4	1.0	9.6
Serum Pool	30	6.60	0.47	7.1

C. Recovery:

One serum sample was spiked with cyanocobalamin and one with MTFA. The MTFA was calibrated by UV spectrophotometry and spiked into the sample. Recoveries were calculated as:

$$\frac{\text{found - endogenous}}{\text{spike}} \times 100$$

Vitamin B_{12}				MTFA			
Spike	Found	Expected	% Recovery	Spike	Found	Expected	% Recovery
0	340	---	---	0	0.9	---	---
200	541	540	100	2	3.03	2.9	107
400	763	740	106	4	4.95	4.9	101
1000	1297	1340	96	10	10.66	10.9	98

D. Linearity

A serum sample was diluted with standard A to test for linearity. Linearity was calculated as:

$$\frac{\text{found}}{\text{expected}} \times 100$$

Vitamin B_{12}				Folate			
Dilution	Found	Expected	%	Dilution	Found	Expected	%
0	398	---	---	0	19.48	---	---
1.33	273	299	91	1.33	14.40	14.6	99
2	195	199	98	2	10.17	9.74	104
4	100	100	101	4	5.49	4.87	113
8	50.0	50	100	8	2.68	2.44	110

E. Specificity

5-Methyltetrahydrofolic acid and pteroylglutamic acid have equal affinity for the binder in this assay.

The porcine Intrinsic Factor used in this kit has been purified by affinity chromatography and contains less than 4% R protein. The purity has been established according to criteria proposed by the National Committee for Clinical Laboratory Standards[16].

The intrinsic factor in the ICN Pharmaceuticals SimulTRAC-SNB Radioassay Kit measures 10,000 pg/mL of cobinamide as less than 75 pg/mL when read against a standard curve constructed from cobalamin standards. In addition, vitamin B_{12} binding is more than 95% inhibited by specific anti-IF blocking antibody.

Additional studies have shown that when using the ICN Pharmaceuticals SimulTRAC-SNB Kit, cobinamide (a vitamin B_{12} analogue) is less than 0.01% cross-reactive.

F. Sensitivity:

Sensitivity as defined by the concentration at 90% trace binding is 75 pg/mL for B_{12} and 0.6 ng/mL for folate.