

K971177

MAY 16 1997

**510(k) Summary of
Safety and Effectiveness**

***This summary of 510(k) safety and effectiveness information is being submitted
in accordance with the requirements of SMDA 1990 and 21 CFR Part 807.92.***

Name: Diagnostic Products Corporation
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Director of Clinical Affairs

Date of Preparation: April 28, 1997

Device Name: IMMULITE® Vitamin B12
Trade: Reagent system for the determination of vitamin
B12 in serum or heparinized plasma

Catalog Number: LKVB1 (100 tests); LKVB5 (500 tests)

Classification: Class II device (862.1810, 75CDD)

Manufacturer: Diagnostic Products Corporation
5700 West 96th Street
Los Angeles, California 90045-5597

Establishment Registration #: DPC's Registration # is 2017183

**Substantially Equivalent
Predicate Device:** DPC's Solid Phase No Boil Dualcount® (K860815);
Bio-Rad's Quantaphase II® B12/Folate Radioassay
(K933315).

Description of Device: IMMULITE® Vitamin B12 is a clinical device for use
with the IMMULITE® Automated Immunoassay
Analyzer.

Intended Use of the Device: IMMULITE® Vitamin B12 is a chemiluminescent
assay for use with the IMMULITE® Automated
Analyzer and designed for the quantitative
detection of vitamin B12 in human serum or
heparinized plasma. It is intended strictly for *in vitro*
diagnostic use as an aid in clinical diagnosis and
treatment of anemia.

Summary and Explanation of the Test:

Vitamin B12 (cobalamin) and folate are nutrients essential to hematopoiesis. Megaloblastic anemia is almost always due to lack of one of these two vitamins. Vitamin B12 deficiency can also result in severe neurological impairment.

Circulating levels of vitamin B12 are usually a good index to tissue stores. That is, vitamin B12 levels as measured in serum or plasma by an optimized assay system are typically low in vitamin B12 deficiency, and normal or elevated otherwise. Exceptions to this rule can occur in those relatively uncommon situations where levels of vitamin B12 transport proteins are abnormal. Thus, low circulating vitamin B12 levels can occur in the absence of vitamin B12 deficiency where the level of transcobalamin I (a physiologically inactive transport protein) is low.

Conversely, vitamin B12 deficiency can occur in the presence of normal or even elevated plasma vitamin B12 levels where transcobalamin II levels are low or where levels of inactive vitamin B12 transport proteins are high, as in chronic myelogenous leukemia. (Circulating folate levels are usually normal or elevated in vitamin B12 deficiency, but red cell folate levels are frequently low in this condition.)

Vitamin B12 deficiency occurs only rarely as a result of dietary lack of this vitamin. More commonly, it results from impaired absorption, as in partial or total gastrectomy, or in pernicious anemia, a condition characterized by absence or near absence of intrinsic factor. Since roughly two thirds of all patients with pernicious anemia have blocking antibodies to intrinsic factor (IFbAb), while IFbAb are only very rarely encountered in other situations, IFbAb determinations represent a useful follow-up test for the differential diagnosis of vitamin B12 deficiency. (Circulating intrinsic factor antibodies are present in more than half of all pernicious anemia patients. Increased transport protein levels can occur, for example in chronic myelogenous leukemia.)

Common causes of high vitamin B12 levels include liver disease, myeloproliferative disease (with chronic myelogenous leukemia as a special case) and the use of multivitamin supplements.

Performance Equivalence - Technology Comparison:

IMMULITE® Vitamin B12 is a chemiluminescent immunoassay, DPC's SPNB Dualcount® is a radioassay. The technology in DPC's IMMULITE® Vitamin B12 is a unique combination of technologies employed in previously cleared and commercially marketed DPC products.

IMMULITE® Vitamin B12 is a chemiluminescent version of the classic method for vitamin B12 radioassay, involving a preliminary heat denaturation step. Vitamin B12 in the patient sample is released from carrier proteins by incubation at 100°C in the

Technology Comparison: *(continued)*

presence of dithiothreitol and potassium cyanide to inactivate intrinsic factor antibodies and even the most extreme levels of vitamin B12 transport proteins.

After the heat denaturation step, the treated patient sample and purified hog intrinsic factor are simultaneously introduced into an IMMULITE® Test Unit containing a polystyrene bead coated with a B12 analog, and incubated for approximately 30 minutes at 37°C with intermittent agitation. During this incubation, vitamin B12 in the treated sample competes with the B12 analog on the solid phase for a limited number of vitamin B12 binding sites on the purified intrinsic factor. (Endogenous vitamin B12 analogs do not interfere, because the binder is free of R-protein.) Alkaline phosphatase-labeled anti-hog intrinsic factor is introduced, and the Test Unit is incubated for another 30-minute cycle. The unbound enzyme conjugate is removed by a centrifugal wash.

Substrate is then added, and the Test Unit is incubated for a further 10 minutes. The chemiluminescent substrate, a phosphate ester of adamantyl dioxetane, undergoes hydrolysis in the presence of alkaline phosphatase to yield an unstable intermediate. The continuous production of this intermediate results in the sustained emission of light, thus improving precision by providing a window for multiple readings. The bound complex - and thus also the photon output, as measured by the luminometer - is inversely proportional to the concentration of vitamin B12 in the sample.

In DPC's SPNB Dualcount® procedure, simultaneous assay of vitamin B12 and folic acid is accomplished by means of a Master Tracer with two isotopes, cobalt 57 (⁵⁷Co) and iodine 125 (¹²⁵I), which are easily separated by most dual-channel gamma counters. Vitamin B12 and folic acid in the patient sample are released from carrier proteins by incubation at an elevated pH, above 12, in the presence of dithiothreitol and potassium cyanide. This inactivates the intrinsic factor antibodies.

Purified hog intrinsic factor and folate binding protein are employed as the binders for vitamin B12 and folic acid, respectively. With the binders immobilized on microcrystalline cellulose particles, isolation of the bound fraction becomes a simple matter of centrifuging and decanting. Counts in the precipitate are then transformed by comparison with a calibration curve into vitamin B12 and folic acid concentrations.

The Quantaphase II® B12 and Folate Radioassays are performed by combining a serum or plasma sample with vitamin B12 (⁵⁷Co) and/or folate (¹²⁵I) in a solution containing dithiothreitol (DTT) and cyanide. The mixture is boiled to inactivate endogenous binding proteins and to convert the various forms of vitamin B12 to cyanocobalamin. The reduced folate and its analogs are stabilized by DTT during the heating procedure which involves placing the patient sample and standards in a boiling water bath at 100°C and allowing the samples to incubate for a minimum of 20 minutes. The mixture is cooled and then combined with immobilized, affinity-purified porcine

Technology Comparison: (continued)

intrinsic factor and folate binding protein. This addition is then incubated for one hour at room temperature.

During incubation, the endogenous and labeled vitamins compete for the limited number of binding sites based on their relative concentrations. The reaction mixture is then centrifuged and decanted. Labeled and unlabeled vitamins binding to the immobilized binding proteins are concentrated at the bottom of the tube in the form of a pellet. The unbound vitamins in the supernatant are discarded and the radioactivity associated with the pellet is counted. Standard curves are prepared using vitamin B12 and folate standards in a human serum albumin base. The concentrations of the vitamin B12 and folate in the serum or plasma sample are determined from the standard curves.

Performance Equivalence - Method Comparison:

The IMMULITE® Vitamin B12 procedure was compared to DPC's SPNB Dualcount® on 98 patient serum samples, with vitamin B12 concentrations ranging from approximately 90 to 1,143 pg/mL.

Means: 385 pg/mL (IMMULITE® Vitamin B12)
 371 pg/mL (DPC's SPNB Dualcount®)

Linear regression analysis of vitamin B12 values yielded the following statistics:

$$\text{(IMMULITE®)} = 0.99 \text{ (SPNB)} + 19.1 \text{ pg/mL}$$
$$r = 0.982$$

The IMMULITE® Vitamin B12 procedure was also compared to Bio-Rad's Quantaphase II® B12 Radioassay on 101 patient serum samples, with vitamin B12 concentrations ranging from approximately 102 to 1,218 pg/mL.

Means: 396 pg/mL (IMMULITE® Vitamin B12)
 412 pg/mL (Quantaphase II® B12)

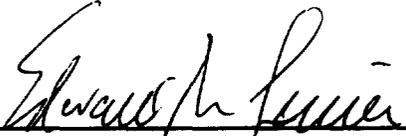
Linear regression analysis of vitamin B12 values yielded the following statistics:

$$\text{(IMMULITE®)} = 0.91 \text{ (Quantaphase II®)} + 19.5 \text{ pg/mL}$$
$$r = 0.965$$

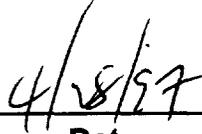
Diagnostic Products Corporation
IMMULITE® Vitamin B12
April 28, 1997

Conclusion:

The data presented in this summary of safety and effectiveness is the data the Food and Drug Administration used in granting DPC substantial equivalence for IMMULITE® Vitamin B12.



Edward M. Levine, Ph.D.
Director of Clinical Affairs



Date