Failures of Cobalamin Assays in Pernicious Anemia

TO THE EDITOR: Cobalamin (vitamin B_{12}) assays have been central to the diagnosis of clinical cobalamin deficiency such as pernicious anemia because the diagnostic sensitivities of older assays have been approximately 95%.[1] However, the competitive-binding luminescence assay (CBLA) replaced older microbiologic and radioisotope-dilution assays during the past decade. Few studies have compared these methods, and cobalamin CBLA has received less-focused scrutiny than older methods have received in the past. In 2000, a study showing that a CBLA failed to detect many low cobalamin levels[2] was disputed by the manufacturer.[3] A later article attributed similar

Table 1. Comparison of Cobalamin Results According to Assay in 23 Cobalamin–Deficient Patients with and without Anti–Intrinsic Factor Antibodies.*

Serum Sample No.	Anti–Intrinsic Factor Antibodies	Radioisotope-Dilution Assay	Competitive-Binding Luminescence Assay			Cause of Cobalamin Deficiency
			No. 1†	No. 2‡	No. 3§	
			cobalamin level — ng/liter			
1	Negative	0	56	94	86	Pernicious anemia
2	Negative	10	65	106	114	Malabsorption of cobalamin in food¶
3	Negative	13	75	72	116	Pernicious anemia
4	Negative	23	20	87	116	Veganism¶
5	Negative	25	0	60	105	Pernicious anemia
6	Negative	25	30	83	106	Postgastrectomy state¶
7	Negative	60	97	167	173	Pernicious anemia
8	Negative	149	155	215	200	Pernicious anemia
9	Positive	0	29	88	103	Pernicious anemia
10	Positive	3	0	57	97	Pernicious anemia
11	Positive	12	239	71	181	Pernicious anemia
12	Positive	17	2	66	129	Pernicious anemia
13	Positive	53	92	141	288	Pernicious anemia
14	Positive	64	123	158	170	Pernicious anemia
15	Positive	88	258	352	313	Pernicious anemia
16	Positive	97	126	185	161	Pernicious anemia
17	Positive	120	126	186	175	Pernicious anemia
18	Positive	127	118	202	206	Pernicious anemia
19	Positive	151	247	234	270	Pernicious anemia
20	Positive	158	268	263	303	Pernicious anemia
21	Positive	162	259	322	306	Pernicious anemia
22	Positive	165	147	216	219	Pernicious anemia
23	Positive	172	188	234	269	Pernicious anemia
Reference interval		190–1016	180–914	223–925	200–700‖	

* Serum levels of cobalamin are expressed in nanograms per liter to conform with usage in almost all clinical laboratories and in clinical practice. To convert the values for cobalamin to picomoles per liter, multiply by 0.738. False normal cobalamin values, based on each assay's reference interval, are underlined.

† The Beckman Coulter Access assay, which used the UniCel DxI 800 Immunoassay System, was performed at New York Presbyterian Hospital.

‡ The Roche Elecsys Systems Modular Analytics E170 instrument and reagents were performed at New York Methodist Hospital.

§ The Siemens Advia Centaur assay was performed at Bellevue Hospital Center, New York. A forerunner of this CBLA was used in earlier studies in which false normal results were observed.[2,4]

¶ The absence of anti–intrinsic factor antibodies is infrequent in patients with pernicious anemia. This patient did not have pernicious anemia, but the results were included to provide a sufficient number of samples from clinically cobalamin-deficient patients without anti–intrinsic factor antibodies.

‖ The reference interval for this assay was established by the clinical laboratory and differs slightly from the manufacturer's recommended interval (211 to 911 ng per liter). Substituting the manufacturer's interval would have changed only one of the diagnoses (in Patient 18).

failure rates with the same CBLA to the diagnostic insensitivity of cobalamin to clinical cobalamin deficiency,[4] without considering assay error.[5] Since 2006, however, five case reports have identified false normal cobalamin levels in seven patients with pernicious anemia (see the Table in the Supplementary Appendix, available with the full text of this letter at NEJM.org), and some authors have proposed that CBLA failure to inactivate serum anti–intrinsic factor antibodies may be responsible.

This highly focused, medically serious assay failure easily eludes routine monitoring, and its extent is unknown. Therefore, we examined three questions: How often does CBLA fail in pernicious anemia? Are many CBLAs affected? Is failure linked to the presence of anti–intrinsic factor antibodies in serum? Because serum samples obtained from untreated patients with pernicious anemia are too scarce for meaningful prospective surveys, we used frozen samples obtained 10 to 15 years ago from 23 untreated patients — 15 with anti–intrinsic factor antibodies and 8 without them. Each patient met five criteria: a low cobalamin level according to a radioisotope-dilution assay, a sufficient volume of the serum used in the radioisotope-dilution assay for additional testing, a clinically expressed cobalamin deficiency, unequivocal proof of pernicious anemia, and a defined anti–intrinsic factor antibody status. Aliquots were tested in three clinical laboratories with the use of different CBLAs; we accepted each laboratory's reference interval in categorizing the results (see the Supplementary Appendix for all methodologic details).

The three CBLAs showed false normal values in 6 of 23 (26%), 5 of 23 (22%), and 8 of 23 (35%) serum samples, respectively, as compared with a radioisotope-dilution assay ($P=0.03$, $P=0.06$, and $P=0.02$) (Table 1). Five serum samples failed with all three CBLAs. False normal results affected 33 to 53% of positive serum samples for anti–intrinsic factor antibodies but no serum samples that were negative for anti–intrinsic factor antibodies ($P=0.01$ to 0.06). The activity of anti–intrinsic factor antibodies was nonsignificantly greater in the 9 serum samples with a cobalamin assay error than in the 6 without an error (93.7% intrinsic factor–blocking activity vs. 88.5%, $P=0.11$). A corrinoid analogue–related assay artifact was ruled out (see the Supplementary Appendix). The study's strengths and limitations are addressed in the Supplementary Appendix.

The diagnostic failures with all three CBLAs suggest widespread CBLA malfunction; indeed, case reports also showed errors in the Immulite 2000 and Siemens Dimension Vista CBLAs (see the Table in the Supplementary Appendix). The reports of errors that began in 2000 suggest that the CBLA problem is also long-standing. Partial solutions are inadequate. Manipulations of cutoff points, which are always problematic,[1] cannot compensate for false normal CBLA levels that often exceed 1000 ng per liter (see the Table in the Supplementary Appendix). CBLA brochures that advise users to test for anti–intrinsic factor antibodies when they are uncertain about a normal cobalamin result ignore the higher priority of preventing the CBLA-specific malfunction in the first place. The advice becomes untenable when assay failure rates are 22 to 35%. Manufacturers, who have access to proprietary information, must instead transparently identify and permanently correct the defect or defects. Control samples containing anti–intrinsic factor antibodies may facilitate monitoring of CBLA improvement.

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