The Elecsys® Vitamin B12 assay is not affected by anti-intrinsic factor auto-antibodies in diagnosis of pernicious anemia

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Abstract

Background

Low Vitamin B12 serum levels in pernicious anemia are often caused by anti-intrinsic factor auto-antibodies which inhibit the uptake of Vitamin B12 by intrinsic factor in the stomach. Two publications in 2012 assert that in-vitro Vitamin B12 competitive-binding luminescence assays (CBLAs) fail in detection of low levels of Vitamin B12 in patients with pernicious anemia because of anti-intrinsic factor auto-antibody interference.[1,2] This interference leads to measurement of a falsely high level of Vitamin B12 and therefore to a medical misclassification of patients with pernicious anemia. Therefore, Roche Elecsys Vitamin B12 assay was examined to show that assay specific pretreatment reagent is efficient in in-vitro denaturation and inactivation of potential interferents like anti-intrinsic factor auto-antibodies what leads to measurement of true B12 levels and therefore to a correct medical classification of patients.

Methods

Anti-intrinsic factor antibody was added to native serum samples in augmenting amounts. Afterwards, serum samples were measured with the Roche Elecsys Vitamin B12 assay - “with pretreatment” and “without pretreatment” on **cobas e** 601 analyzer. To sustain Vitamin B12 pipetting scheme, pretreatment was replaced by H_2O in measurements “without pretreatment”.

Results

Without pretreatment, an interference caused by addition of anti-intrinsic factor antibody is obvious. Interference is increasing with augmenting concentration of antibody. With pretreatment, which is part of the standard Elecsys B12 assay procedure, interference by anti-intrinsic factor antibody is avoided.

Methods

Roche Elecsys Vitamin B12 assay was examined to show that assay specific pretreatment reagent is efficient in in-vitro denaturation and inactivation of potential interferents – like anti-intrinsic factor auto-antibodies – what leads to measurement of true B12 levels and therefore to a correct medical classification of patients.

Anti-intrinsic-factor antibody (Abcam [29/011] [ab128402]) was added to native serum samples in augmenting amounts. Serum samples were measured with the Elecsys Vitamin B12 assay- “with pretreatment” and “without pretreatment” on **cobas e** 601 analyzer (n=2). To sustain Vitamin B12 pipetting scheme, pretreatment was replaced by H_2O in measurements “without pretreatment”. Signal obtained in measurements is expressed in Relative Light Units (RLU).

Measurement of Vitamin B12 with pretreatment (standard assay procedure)

1st incubation:
Alkaline pretreatment step releases the bound vitamin B12 from endogenous binding proteins. Binding proteins and anti-IF-AB are immediately denatured at the high pH.

2nd incubation:
Pretreated sample is incubated with the ruthenium labeled IF. A vitamin B12~IF complex is formed, the amount of which is dependent upon the analyte concentration in the sample.

Detection:
Streptavidin-coated microparticles and vitamin B12 labeled with biotin are added, and the still-vacant sites of the ruthenium labeled IF become occupied, with formation of a ruthenium labeled IF~vitamin B12 biotin complex (competitive assay principle).

Result is reflecting true B12 concentration in patient sample.

Measurement of Vitamin B12 without pretreatment

1st incubation:
Pretreatment reagents were replaced with dest. H_2O
- No release of bound vitamin B12 from endogenous binding proteins
- No denaturation of IF and anti-IF-AB

2nd incubation:
Anti-IF-Ab binds to the ruthenylated intrinsic-Factor (Ru-IF).

Detection:
Result is impacted by anti-IF-AB interference.

False high B12 concentration (=low RLU) due to anti-IF-AB interference.

Results

Without pretreatment, an interference caused by addition of anti-intrinsic-factor antibody is obvious. Interference is increasing with augmenting concentration of antibody. Decreasing RLU suggest a binding of the anti- intrinsic-factor antibody to ruthenylated intrinsic factor (IF-Ru) what inhibits binding of biotinylated B12 (B12-Bi). With pretreatment, interference by anti-intrinsic factor antibody is avoided.

Saturation of inhibitory effect was reached with 200 µg/mL antibody and no further significant decrease in measured RLU can be achieved by further increase of antibody concentration (Figure 2). Recovery of measured RLU after addition of anti-intrinsic-factor antibody was calculated. Samples without antibody added (Figure1) or equal volume of PBS (phosphate buffered saline) solution added (Figure 2) were chosen as reference in recovery calculation for all antibody concentrations.

1. Anti-Instrinsic Factor antibody (Acris)

		With Pretreatment		Without Pretreatment	
	Antibody-Conc µg/mL	RLU	% Recovery Reference = 0 g/mL antibody	RLU	% Recovery Reference = 0 g/mL antibody
HS-1	0	80339	100 %	126783	100 %
	1	79665	99 %	113433	89 %
	5	79300	99 %	100159	79 %
	20	80063	100 %	90469	71 %
	100	83336	104 %	77553	61 %
HS-2	0	82859	100 %	132297	100 %
	1	82106	99 %	116206	88 %
	5	81915	99 %	102916	78 %
	20	81699	99 %	92543	70 %
HS-3	0	85060	100 %	133215	100 %
	1	83374	98 %	118603	89 %
	5	82143	97 %	105526	79 %
	20	84728	100 %	94000	71 %

2. Anti-Instrinsic Factor antibody (Abcam)

		With Pretreatment		Without Pretreatment	
	Antibody-Conc µg/mL	RLU	% Recovery Reference = 0 g/mL antibody	RLU	% Recovery Reference = 0 g/mL antibody
HS-1	0	80339	100 %	126783	100 %
	1	79193	99 %	113783	90 %
	5	79425	99 %	98608	78 %
	20	79781	99 %	88533	70 %
	100	83695	104 %	77108	61 %
HS-2	0	82859	100 %	132297	100 %
	1	80327	97 %	115650	87 %
	5	81374	98 %	101679	77 %
	20	81376	98 %	92154	70 %
HS-3	0	85060	100 %	133215	100 %
	1	83268	98 %	117937	89 %
	5	81538	96 %	104952	79 %
	20	82955	98 %	93609	70 %

Figure 1: Measurement of serum samples spiked with Anti-Instrinsic Factor antibody (Acris BM 551, Abcam [29/011] [ab128402]). Recovery of measured RLU after addition of anti- intrinsic-factor antibody was calculated. Samples without addition of antibody were chosen as reference in recovery calculation.

Anti-Instrinsic Factor antibody (Abcam)

		With pretreatment			Without pretreatment		
	Antibody-Conc µg/mL in case of antibody added	RLU	RLU	% Recovery Reference = accordant sample with PBS added	RLU	RLU	% Recovery Reference = accordant sample with PBS added
anti-IF-antibody		+	–		+	–	
PBS		–	+		–	+	
HS	0	86380	79948	108	144628	142422	102
	50	88984	81030	110	94930	141042	67
	100	89534	84688	106	87657	141663	62
	200	93856	87599	107	80162	140818	57
	300	96525	90637	106	77052	141213	55
	400	99783	97130	103	74827	140769	53

Figure 2: Titration of inhibitory effect with Anti-Instrinsic Factor antibody (Abcam [29/011] [ab128402]). Recovery of measured RLU after addition of anti-intrinsic-factor antibody was calculated. Samples with equal volume of PBS (phosphate buffered saline) solution added were chosen as reference in recovery calculation for all antibody concentrations. “+” means addition of anti-IF-antibody or PBS respectively ; “–” means no addition of anti-IF-antibody or PBS respectively

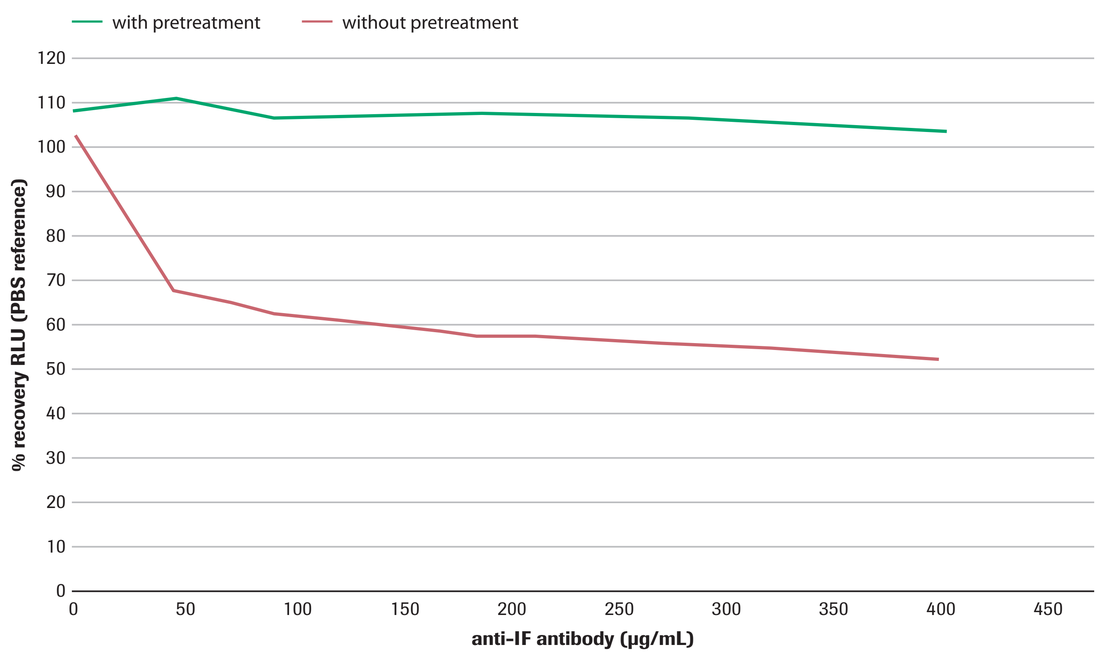

Figure 3: Graphical chart of results shown in Figure 2. The x axis depicts concentration of anti-intrinsic-factor antibody added; the y axis depicts recovery of RLU in samples with anti-intrinsic-factor antibody added in comparison to samples with PBS added.

Conclusion

These experiments clearly show that the Elecsys Vitamin B12 assay is not affected by anti-intrinsic factor auto-antibodies in diagnosis of pernicious anemia. The formulation of the assay specific pretreatment reagent ensures complete in-vitro denaturation and inactivation of potential interferents like anti-intrinsic factor auto-antibodies.

References
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