SIEMENS

ADVIA Centaur® XP

Siemens Healthcare Diagnostics Inc.

Customer Bulletin

February 2016

Introducing the Active-B12 Assay (AB12) (OUS Only)

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Introduction

Siemens Healthcare Diagnostics is pleased to announce the availability of the newly developed Active-B12 (Holotranscobalamin) for use on the ADVIA Centaur® XP system.

Table 1. Product Ordering Information

Product Name	Product Description	Siemens Material Number (SMN)
ADVIA Centaur AB12	ReadyPack/100 tests	10995088

Please retain this bulletin with your records for future reference.

Background Information

Vitamin B12 (cobalamin) in serum is bound to two proteins: transcobalamin (TC) and haptocorrin (HC). The transcobalamin-vitamin B12 complex is called Holotranscobalamin (HoloTC). HoloTC contains the biologically available cobalamin as only HoloTC promotes the uptake of cobalamin by all cells via specific receptors. In comparison, approximately 80% of the circulating cobalamin that is carried by haptocorrin is considered metabolically inert because no cellular receptors exist, with the exception of receptors found in the liver.

Genetic absence of haptocorrin is rare and not considered a serious condition. Genetic absence or abnormalities of transcobalamin, however, manifest as typical hematological, neurological, and metabolic pathologies of cobalamin deficiency, which require aggressive treatment even if a serum analysis results in normal cobalamin concentrations.

The shorter circulating half-life of HoloTC compared to holohaptocorrin (HoloHC) makes a decrease of HoloTC one of the earliest markers of cobalamin deficiency.[1]

The measurement of total serum cobalamin suffers from some limitations; in particular, most of the cobalamin that is measured is bound to haptocorrin. A number of studies have been published to support that HoloTC would be a better indicator of vitamin B12 status than total serum cobalamin.[2, 3] Methods based on specific anti- transcobalamin antibodies have been available and confirm the usefulness of HoloTC for diagnosing B12 deficiency. As expected, HoloTC levels are low in patients with biochemical signs of vitamin B12 deficiency.[4] Notably, low values have been reported in vegetarians, [5] vegans, [6] and in populations with low intake of vitamin B12.[7] In addition, low levels of HoloTC (but not total vitamin B12) in serum were reported in patients with Alzheimer's disease compared to levels of HoloTC in a healthy control group.[8] HoloTC levels reflect vitamin B12 status, independent of recent absorption of the vitamin.[9]

Siemens Healthcare Diagnostics Inc., Tarrytown, NY 10591-5097 USA

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Availability

The ADVIA Centaur XP AB12 assay will be implemented beginning with lot 26096003, expected to ship in February 2016.

Key Features

- Longer onboard stability and calibration interval to help minimize reagent waste and control costs.
- Increased ability for workload consolidation to improve efficiency and staff utilization.

Intended Use

The ADVIA Centaur Active-B12 (Holotranscobalamin) (AB12) assay is for *in vitro* diagnostic use in the quantitative measurement of holotranscobalamin (HoloTC) in human serum using the ADVIA Centaur XP system. Active-B12 (Holotranscobalamin) is used in the diagnosis and treatment of vitamin B12 deficiency.

Product Information Overview

Table 2. Product Information Overview

Item	Description
Reagent SMN 10995088 ReadyPack® includes primary reagent pack Lite Reagent and Solid Phase Reagent AB12 Calibrator	ADVIA Centaur AB12 Quantity 100 tests ADVIA Centaur systems AB12 Master Curve Card Reagents are all liquid, ready to use Calibrators are included with each kit: • 1 vial AB12 ADVIA Centaur low calibrator • 1 vial AB12 ADVIA Centaur high calibrator ADVIA Centaur systems AB12 Calibrator Assigned Value card; liquid, ready to use
Diluent SMN 10492364 ReadyPack reagent pack	ADVIA Centaur Multi-Diluent 13 Quantity 10.0 mL/pack Provided for automatic dilutions of patient samples
ADVIA Centaur Wash 1 REF # 01137199 or 03773025 ReadyPack reagent pack	2 x 1500 mL/pack or 2 x 2500 mL/pack
Quality Control SMN 10995091	ADVIA Centaur Active-B12 Quality Control Material Quantity 2 levels, 7 mL/bottle ADVIA Centaur Systems AB12 lot-specific value sheet

Item	Description
Master Curve Materials (MCM) SMN 10995090	ADVIA Centaur AB12 MCM 5 x 2.0 mL ADVIA Centaur Systems AB12 lot-specific value sheet
Method Principle	Two-step immunoassay – Antibody-Capture Format - chemiluminescent technology
Measuring Interval	5.00 – 146.00 pmol/L
Detection Limits	Limit of Quantitation (LoQ): 5.00 pmol/L
Specimen Types	Serum
Sample Storage	At 20–25° C: 16 hours separated specimens At 2–8° C: 3 days separated specimens Frozen at -20° C: 3 months and 1 freeze/thaw cycle
Sample Volume	50 µL
Reagent Storage	2–8°C
Reagent Stability at 2–8°C (shelf-life, unopened)	Stable until the expiration date on product
Reagent On-System Stability	44 days
Calibration Interval	44 days
Software Requirements	XP 5.2.3.1SPS and higher & 7.1.1.2 and higher
Required Test Definitions	TDef 1.0.DJ/DK (SMN 11219255/11219259)
Turnaround Time to First Result	47 minutes

Calibration

Traceability of Standardization

The ADVIA Centaur AB12 assay is traceable to an internal standard. Currently there is no reference standard for this assay. Assigned values for calibrators are traceable to this standardization.

Calibration Verification or Measuring Interval Validation Requirements

ADVIA Centaur Active-B12 (AB12) Master Curve Material (MCM) is available for use on the ADVIA Centaur XP system. Refer to Table 2 for a summary of specifics for the ADVIA Centaur AB12 Master Curve Material (MCM).

The AB12 MCM will assist in the documentation of calibration and reportable range verification required by regulatory agencies. MCM targets and ranges are provided on the lot-specific value sheet.

MCMs are traceable to the AB12 internal standardization.

Run quality control material to further assess the acceptability of the calibration.

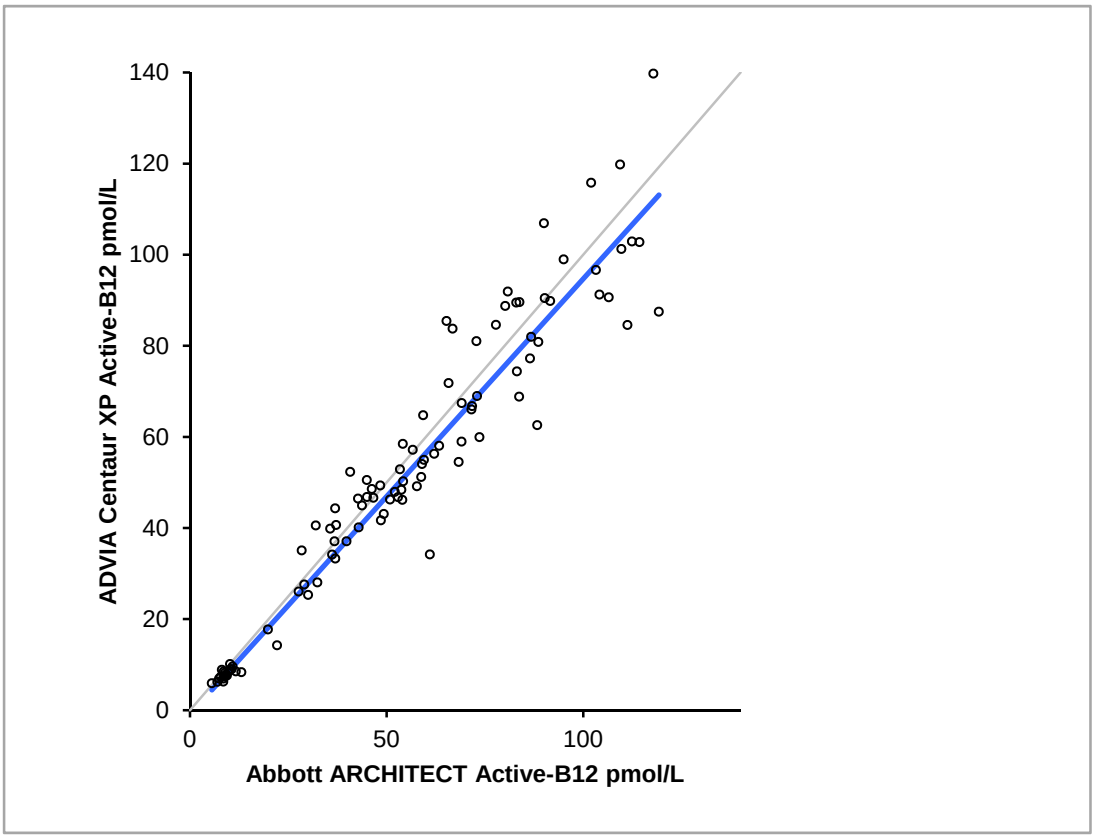
Performance Data

Method Comparisons

Method comparison testing of ADVIA Centaur AB12 assay was conducted in accordance with CLIA EP09-A2[10]: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline, second edition. Customers may observe slightly different data due to inherent inter-laboratory variability.

The ADVIA Centaur AB12 assay has been compared with the Abbott ARCHITECT Active-B12 assay. Refer to Figure 1.

Figure 1. ADVIA Centaur XP Active-B12 vs. Abbott ARCHITECT Active-B12



Parameter	Result
Slope	0.96
Intercept pmol/L	-0.85
Correlation Coefficient (r)	0.96
n	100
Range pmol/L	6.26 – 139.79

Specimen Collection Comparison

Serum tube type results using Active-B12 on the ADVIA Centaur XP system are equivalent based on the following ordinary least squares regression. Serum separator tubes were compared to plain serum tube types.

Table 3. Serum Separator Tubes vs. Plain Serum Tube Types

Sample Type: Serum	Result
Slope	1.01
Intercept pmol/L	-0.46
Correlation Coefficient (r)	1.00
n	48
Range pmol/L	24.00 – 143.44

Linearity

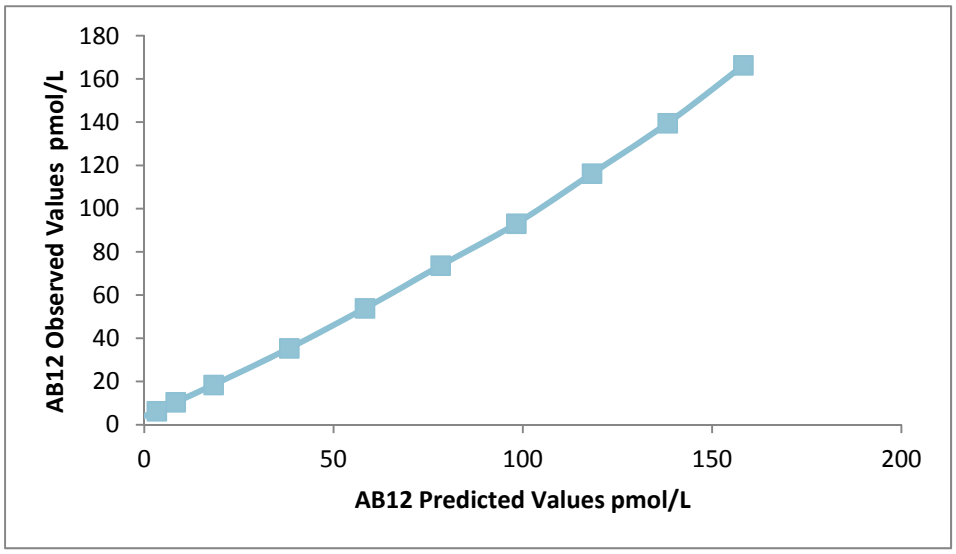
Linearity was evaluated according to the CLSI protocol EP06-A[11]. Two samples containing high levels of active-B12 were mixed with a pool of artificial serum matrix. The resulting sample mixtures were assayed for active-B12. The ADVIA Centaur AB12 assay is linear from 5.00 – 146.00 pmol/L.

Table 4. Observed vs. Predicted

Level	Observed (y) (pmol/L)	Predicted* (x) (pmol/L)	Difference (y-x)	Difference %
1	2.87	-1.68	4.55	NA
2	6.30	3.32	2.98	NA
3	10.42	8.32	2.1	NA
4	18.47	18.31	0.16	1%
5	35.39	38.31	-2.92	8%
6	53.89	58.3	-4.41	8%
7	73.68	78.29	-4.61	6%
8	93.06	98.28	-5.22	5%
9	116.25	118.27	-2.02	2%
10	139.57	138.27	1.3	1%
11	166.33	158.26	8.07	5%

*Predicted values were calculated from a weighted linear regression analysis.

Figure 2. Dilution Linearity



Parameter	Result
Slope	0.98
Intercept pmol/L	-4.49
Correlation Coefficient (r)	0.99

Extended Measuring Range

The extended measuring range is the range of analyte values that may be reported after allowing for specimen dilution to extend the analytical measurement range.

The AB12 assay requires automatic dilution of patient samples with Multi-Diluent 13 (SMN 10492364). To obtain the Extended Measuring Range, the system multiplies the upper limit of the Analytical Measurement Range (AMR) by the AB12 recommended verified dilution factor of 2.

146.00 pmol/L x 2 = 292.00 pmol/L

Extended Measuring Interval for AB12: 5.00 – 292.00 pmol/L

Many patients take B12 dietary supplements. There are no indications of toxic levels of B12 from taking supplements. Normal healthy active-B12 results may be above the analytical measurement range (146.00 pmol/L) and would require automatic dilutions to report patient results.

Hemolysis, Icterus, Lipemia (HIL) Interference

Less than 10% interference with the assay was observed in the presence of the following:

- 500 mg/dL hemoglobin
- 60 mg/dL of unconjugated bilirubin
- 40 mg/dL of conjugated bilirubin
- 1000 mg/dL of lipemia (intralipid)

Performance Characteristics

For all performance characteristic information, refer to the following ADVIA Centaur XP AB12 IFU:

- ADVIA Centaur XP IFU Active B-12 (AB12), RPBL1160/R1_Rev. A, 2016/01 (English).

Regulatory Classification and Reimbursement Codes

Table 5. Important Codes

Item	Description
CLIA Complexity Category	Moderately complex under the US CLIA 1988 regulations.
LOINC® (Logical Observation Identifier Names and Codes)	72160-5 Holo-transcobalamin II [Moles/volume] in Serum
Proficiency Tests	**Description**
College of American Pathologists (CAP)	No known program is available Instrument code: 3211 ADVIA Centaur XP system Method Master list: 1695 Chemiluminescence
National External Quality Assessment Service (NEQAS) pilot program	Code by Manufacturer: Siemens Behring or Centaur – SMS Diag. Ltd Code by method: Chemiluminescence Code by test: ADVIA Centaur AB12 assay
Reference Institute for Bioanalytics (DGKL)	No known program is available Code by Producers: 40 Siemens Healthcare (ADVIA/Chem. Bayer Diag.) Method code: 4 luminescence detection
Royal College of Pathologists Association (RCPA)	No known program is available Instrument code: ADVIA Centaur XP system Method code: Chemiluminescence

Quality Control

Reporting AB12 QC Results to Your QC Program Provider

Table 6. Reporting AB12 QC Results

Item	Description
Analyte	Active-B12 (Holotranscobalamine)
Method	Chemiluminescence
Instrument	Siemens ADVIA Centaur systems
Reagent	Active-B12 (AB12)
Units [SI]*	pmol/L

*Système International d'Unités [SI Units]

QC Results Obtained by Siemens for the AB12 Assay

ADVIA Centaur AB12 QC (SMN 10995091) is available for use with the new ADVIA Centaur AB12 assay. This QC part number is valued assigned for use with the ADVIA Centaur AB12 assay (SMN 10995088) reagent kits.

The means and ranges were derived from replicate analyses using available AB12 reagent lots and ADVIA Centaur AB12 calibrators. These ranges should be used as guidelines.

Each laboratory should establish its own ranges based on multiple lots of AB12 reagent and kit calibrator. Variations over time and between laboratories may be caused by differences in laboratory technique, instruments, reagent lots and QC product.

Commercial Controls

Commercial controls may not be available for this assay at this time. When available, commercial quality control product QC ranges from other manufacturers will need to be established by individual users.

Proficiency Testing (PT)

Siemens has verified the compatibility of the ADVIA Centaur AB12 assay on the ADVIA Centaur XP system with the National External Quality Assessment Service (NEQAS) Active B12 "Serum Holotranscobalamin" pilot survey for Active-B12.

There are no other PT providers with a program for Active-B12 at this time. Follow your local regulatory and accreditation requirements for meeting proficiency testing needs for Active-B12.

Regulatory Information

Assay availability is subject to local regulatory requirements and, therefore, varies by country. If you have any questions or need additional information, please contact your local technical support provider or distributor.

Frequently Asked Questions

Question	Answer
What is the reagent lot-to-lot variability for the AB12 assay?	This assay is designed to meet a ±6.5% recovery bias or ≤ 3 pmol/L (whichever is greater) lot-to-lot variability.
Will the manual dilution option be available with Multi-Diluent 13?	No, only onboard (auto) dilutions are available with the Multi-Diluent 13 ancillary pack reagent (SMN 10492364).
Are other commercial controls available for use with this assay? For example, Bio-Rad controls?	Other commercial controls are not available.
Are optional units available with the AB12 assay?	No, only the SI pmol/L units have been defined and verified for this assay.

References

1. Nexo E. Hvas A-M. Bleie Ø *et al*. Holo-transcobalamin is an early marker of changes in cobalamin homeostasis. A randomized placebo-controlled study. *Clin Chem* 2002;48(10):1768-71.
2. Valente E. Scott JM. Ueland PM *et al*. Diagnostic accuracy of holotranscobalamin, methylmalonic acid, serum cobalamin, and other indicators of tissue vitamin B12 status in the elderly. *Clin Chem* 2011;57(6):856-863.
3. Nexo E. Hoffmann-Lucke E. Holotranscobalamin; a marker of vitamin B12 status: analytical aspects and clinical utility. *Am J Clin Nutr* 2011;94(1):359S-365S.
4. Obeid R. Jouma M. Hermann W. Cobalamin status (holotranscobalamin, methylmalonic acid) and folate as determinants of homocysteine concentration. *Clin Chem* 2002;48(11):2064-5.
5. Herrmann W. Schorr H. Obeid R *et al*. Vitamin B12 status. particularly holotranscobalamin II and methylmalonic acid concentrations and hyperhomocysteinemia in vegetarians. *Am J Clin Nutr* 2003;78:131-6.
6. Lloyd-Wright Z. Hvas A-M. Moller J *et al*. Holotranscobalamin as an indicator of dietary vitamin B12 deficiency. *Clin Chem* 2003;49(12):2076-8.
7. Refsum H. Yajnik CS. Gadkari M *et al*. Hyperhomocysteinemia and elevated methylmalonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians. *Am J Clin Nutr* 2001;74:233-41.
8. Refsum H. Smith AD. Low Vitamin B12 status in confirmed Alzheimer's disease as revealed by serum holotranscobalamin. *J Neurol Neurosurg Psychiatry* 2003;74:959-61.

9. Chen X. Remacha AF. Sarda MP *et al*. Influence of cobalamin deficiency compared with that of cobalamin absorption on serum holo-transcoabalamin II. *Am J Clin Nutr* 2005;81:110-14.
10. Clinical and Laboratory Standards Institute (CLSI). *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition (Interim Revision).* CLSI document EP09-A2-IR [ISBN 1-56238-731-6]. Wayne, PA: CLSI; 2010.
11. National Committee for Clinical Laboratories Standards. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. NCCLS Document EP06-A National Committee for Clinical Laboratories Standards; Wayne, PA 2003.

Additional Assistance

Technical information is available at http://www.siemens.com/document-library. If you need additional assistance, please contact your Siemens Customer Care Center.

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