



Laboratory Procedure Manual

Analyte: **Vitamin B12 (B12)**

Matrix: **Serum**

Method: **Roche E-170 Vitamin B12 “ECLIA”**

Method No: 4009.02

Revised:

As performed by:

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Important Information for Users

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing items listed.

Data File Name	Variable Name	SAS Label
VB12_G	LBXB12	Vitamin B12 (pg/mL)

1. Summary of Clinical Relevance and Test Principle

A. Clinical relevance

Vitamin B₁₂ (B12) is an essential cofactor for two enzymes involved in one-carbon metabolism: methylmalonyl CoA mutase (reduced function of this enzyme results in increased serum methylmalonic acid (MMA) levels) and methionine synthetase (this enzyme catalyzes the remethylation of homocysteine to methionine) [1]. A serum B12 level below the normal expected range may indicate B12 deficiency. However, a B12 level within the low normal range does not exclude B12 deficiency; symptomatic patients need to be further evaluated with MMA, folic acid, and homocysteine [2,3].

A chronic dietary deficiency of either folate or vitamin B12 causes macrocytic anemia, although strict dietary deficiencies are rare. Most people who develop a vitamin B12 deficiency have an underlying stomach or intestinal disorder that limits the absorption of vitamin B12. Subtly reduced cognitive function resulting from early vitamin B12 deficiency is sometimes the only symptom of these intestinal disorders. Untreated deficiencies will lead to megaloblastic anemia and vitamin B12 deficiency results in irreversible central nervous system degeneration. Hematologic signs, however, are not always present in vitamin B12 deficiency and hematologic signs and neurologic abnormalities can be inversely correlated [4].

B. Test principle

The Elecsys Vitamin B12 assay employs a competitive test principle using intrinsic factor specific for vitamin B12. The fully automated electrochemiluminescence immunoassay "ECLIA") is intended for use on Elecsys and cobas e immunoassay analyzers. The total duration of the assay is 27 minutes. The 1st step is to incubate 15 uL of sample with the vitamin B12 pretreatment 1 and pretreatment 2 to release bound vitamin B12. The 2nd incubation adds the ruthenium labeled intrinsic factor to the pretreated sample causing a vitamin B12-binding protein complex to form; the amount of which is dependent upon the analyte concentration in the sample. During the 3rd incubation, streptavidin-coated microparticles and vitamin B12 labeled with biotin are added and the still-vacant sites of the ruthenium labeled intrinsic factor become occupied. This forms a ruthenium labeled intrinsic factor-vitamin B12 biotin complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve which is instrument specifically generated by a 2 point calibration and a master curve provided via the reagent barcode [5].

2. Safety Precautions

Consider all specimens potentially positive for infectious agents including HIV, hepatitis B and hepatitis C. We recommend that the hepatitis B vaccination series for all the analysts working with whole blood and/or serum. Observe universal precautions; wear protective gloves, laboratory coats, and safety glasses during all steps of this method. Discard any residual sample material by autoclaving after analysis is completed. Place all disposable plastic, glassware, and paper (pipette tips, vials, gloves, etc.) in a biohazard autoclave bag and keep these bags in appropriate containers until sealed and autoclaved. Wipe down all work surfaces with 10% bleach solution when work is finished.

Handle acids and bases with extreme care; they are caustic and toxic. Handle organic solvents only in a well-ventilated area or, as required, under a chemical fume hood.

Reagents and solvents used in this study are listed in Section 6. Material safety data sheets (MSDSs) for all chemicals are readily accessible as hard copies in the lab. If needed, MSDS for other chemicals can be viewed at <http://www.ilpi.com/msds/index.html> or at <http://intranet.cdc.gov/ohs>.

3. Computerization and Data System Management

- A. During sample preparation and analysis, samples are identified by their sample ID. The sample ID is a number that is unique to each sample that links the laboratory information to demographic data recorded by those who collected the sample.
- B. Calculation of B12 concentration is accomplished with the software on the Roche E-170 and generated data is copied onto a floppy disk as an ASCII file and transferred to the DLS network where it is saved. The results file is imported into a database for review of the patient data, statistical evaluation of the QC data, and approval of the results. See **“SOP Computerization and Data System Management”** for a step-by-step description of data transfer, review and approval.
- C. NHANES data is transmitted electronically on a regular basis (approximately weekly for 3-week turnaround analytes). Abnormal values are confirmed by the analyst, and codes for missing data are entered by the analyst and are transmitted as part of the data file. NCHS makes arrangements for the abnormal report notifications to the NCHS Survey Physician.
- D. The data file and results file from the instrument workstation are typically backed up daily to the Roche/Hitachi USB Memory Stick for long-term storage. This is the responsibility of the analyst under the guidance of the project lead person. Files stored on the DLS network are automatically backed up nightly by ITSO support staff.

4. Specimen Collection, Storage, and Handling Procedures: Criteria for Specimen Rejection

- A. For best results, a fasting sample should be obtained, but fasting is not required.
- B. Specimens for B12 analysis may be fresh or frozen serum or plasma. Serum specimens may be collected with regular red-top Vacutainers or tubes containing separating gel and plasma specimens may be collected with Na-heparin or K₃-EDTA as an anticoagulant.
- C. A routine test requires 150 µL for the sample cup. For every additional B12 test performed from the same container, an additional 15 µL of samples is required. Thus, a 500 µL serum sample is preferable to allow for repeat analyses. The appropriate amount of serum is dispensed into a Nalge cryovial or other plastic screw-capped vials labeled with the participant's ID.
- D. Specimens collected in the field should be kept cold and protected from light. After processing, specimens should be frozen and shipped on dry ice by overnight mail. Once received, they should be stored at <-20°C until analyzed. For long term storage, specimens should always be frozen at -70°C. Serum B12 is fairly stable at -20°C to -70°C and it can withstand 3 freeze/thaw cycles. Refrigerated samples may be used if they are kept cold and brought promptly (within 2 hours) from the site of blood collection. Protect from light.
- E. Ensure that the patients' samples, calibrators and controls are at ambient temperature (20-25°C) before measurement. Once the samples, calibrators, and controls are loaded on the analyzers, they should be measured within 2 hours because of possible evaporation effects.
- F. Specimen handling conditions are outlined in the DLS Policies and Procedures Manual. The protocol discusses collection and transport of specimens and the special equipment required. If there is more than one analyte of interest in the specimen and it needs to be divided, the appropriate amount of blood, serum or plasma should be transferred into a sterile Nalge cryovial labeled with the participant's ID.

5. Procedures for Microscopic Examinations: Criteria for Rejection of Inadequately Prepared Slides

Not applicable for this procedure.

6. Preparation of Reagents, Calibration (Standards), Controls, and All Other Materials: Equipment and Instrumentation

A. Reagent Preparation

All reagents are supplied by Roche Diagnostics in a ready-for-use unit that cannot be separated. Store the reagent kit **upright** in order to ensure complete availability of the microparticles. Bring the cooled reagents to approximately 20°C (45 minutes at room temp) and open the lids slightly before placing on the reagent disk of the analyzer. The reagent kit is stable until the expiration date or up to 12 weeks at 2-8°C after opening, whichever comes first. **The B12 reagent pack can only be stored on-board the E-170 for a maximum of 5 weeks** so the reagent pack is generally removed from the instrument and stored at 2-8°C when all samples are completed.

B. Standards Preparation

Elecsys Vitamin B12 CalSet II is supplied by Roche Diagnostics [6]. Dissolve carefully the contents of one bottle each of B12 Cal1 and B12 Cal2 by adding exactly 1.0 mL of distilled water to each. Allow to stand closed for 15 minutes to reconstitute. Mix carefully, avoiding the formation of foam. Transfer aliquots of the reconstituted calibrator into the empty labeled snap-cap bottles (CalSet Vials). Attach the supplied labels to the additional bottles. Perform only one calibration procedure per aliquot. Store the remaining CalSet vials at 2-8°C for 3 days or at -20°C for 3 months (freeze only once).

C. Preparation of Quality Control Materials

1) Roche controls:

Elecsys PreciControl Varia 3 can be used for quality control of the Vitamin B12 immunoassay on the E-170 analyzer [7]. This is a lyophilized control serum based on human serum matrix in three concentration ranges. The lot specific values need to be entered into the Mod PE before analysis. Carefully dissolve the contents of one bottle of each level by adding exactly 3.0 mL of distilled water to each and allow to stand closed for 30 minutes to reconstitute. Mix carefully, avoiding the formation of foam. Transfer aliquots of the reconstituted controls into empty snap-cap bottles (ControlSet Vials). Attach the supplied labels to these additional bottles. Aliquots intended for storage should be frozen immediately at -20°C and are stable for 1 month (freeze only once). Controls stored at 2-8°C are stable for 3 days. Ensure the controls are at ambient temperature before measurement.

2) CDC QC pools:

Quality control materials for this assay are prepared in-house from blood products acquired from blood banks or from other volunteer blood donors. After screening the pools for Vitamin B12, the serum is pooled to obtain the desired QC levels. All pools are filtered through gauze to remove debris before being dispensed. Serum (usually 750 µL) is aliquoted into labeled 2.0-mL Nalge cryovials, capped, and stored at -70°C. The QC pools are stable for at least 3 years.

The QC limits for all pools are established by analyzing duplicates of each pool for at least 20 consecutive runs.

D. Other Materials

The following materials are available from the manufacturer (Roche Diagnostics):

- 1) Sample racks
- 2) Sample cups (Standard)
- 3) ProCell M system buffer
- 4) CleanCell measuring cell cleaning solution
- 5) PC/CC-Cups to prewarm ProCell M and CleanCell M
- 6) ProbeWash M cleaning solution for finalization and rinsing
- 7) PreClean M detection cleaning solution
- 8) Assay Tip/AssayCup Combimagazine M (reaction vessels and pipette tips)
- 9) Wasteliner
- 10) SysClean system cleaning solution

E. Instrumentation

- 1) MODULAR ANALYTICS E170[®] system (Roche Diagnostics, Indianapolis, IN)
- 2) Daigger Vortex Genie 2 (VWR, Suwanee, GA)
- 3) Eppendorf micropipet and tips (Brinkmann Instruments Co., Westbury, NY)

7. Calibration and Calibration Verification Procedures

For commercial kit assays, calibration procedures recommended by the manufacturer are followed.

Every Elecsys Vitamin B12 reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys Vitamin B12 CalSet II. Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

- every 28 days when using the same **reagent lot**
- every 7 days if using the same **reagent kit**.
- as required: e.g. if quality control findings are outside the specified limits

Please refer to the Roche *Modular Analytics[®] Operator's Manual* and the "**SOP Modular PE Calibration**" for additional details.

Calibration verification is conducted at least twice a year using international reference materials. For details, see **4009_SOP Calibration and Calibration Verification B12**.

In 2005, the National Institute of Standards and Technology (NIST) released a new three level standard reference material for homocysteine and folate in human serum, **SRM 1955**. This material can be used for calibration verification [8]; however, this material only has information B12 values from the Bio-Rad assay and no certified B12 values.

In 2006, the National Institute for Biological Standards and Control (NIBSC) issued a lyophilized, one level reference material for folate and vitamin B12, **NIBSC 03/178**; 2nd International Standard for Folate, Vitamin B12, Recombinant [9]. This material, when reconstituted, has an assigned value of 480 pg/mL (consensus value). Serial dilutions can be made to check accuracy and linearity.

Calibration can also be verified by running the Elecsys Vitamin B12 CalCheck as unknowns.

The laboratory participates in two external proficiency testing programs:

- twice a year in the CAP LN5 Ligand calibration verification / linearity surveys
- three times a year in the CAP K General Ligand – Endocrinology surveys
- twice a year in the UK NEQAS Haematinics survey

Method figures of merit are presented in **Appendix 1**.

As this assay must be performed according to the manufacturer's specifications, none of the parameters can be altered. Therefore, ruggedness testing cannot be performed for this assay.

8. Procedure Operating Instructions, Calculations, and Interpretation of Results

A. Preliminaries

- 1) Allow Calibrators, QC and patient samples to reach ambient temperature.
- 2) Ensure that the amount of reagents, diluent, and wash solutions are adequate for the amount of samples to be run. You may place more than one bottle of reagent at a time on the analyzer; however, avoid using more than one lot number of reagent for a single run.
- 3) Make sure the analyzer and/or tests required are not masked.
- 4) Check to see if calibration is required for the tests that will be run.
- 5) If running the same tests on all samples, go to the "Start" global button and set the "default profile".
- 6) Be sure to clear all previously programmed samples from the Data Review screen after backing up the data.
- 7) Perform the required maintenance on the E-170 system.

B. Instrument Maintenance

The E-170 system maintenance consists of daily, weekly, 2 week and as needed maintenance [10].

- 1) Daily maintenance should be performed at the start of each 8-hour shift, or more frequently, if necessary.
- 2) For additional maintenance requirements, refer to the instrument maintenance logs. For detailed, step by step instructions, refer to the Roche *Modular Analytics*® *Operator's Manual*.

C. Preparing the Run

One run is defined as 100 samples or less.

Each run must contain CDC Vitamin B12 QC pools at the beginning of the run before patient samples are run and at the end of the designate run.

When performing small runs or confirmation (repeat) runs, all levels of CDC Vitamin B12 QC pools must be run in duplicate.

NOTE: Before starting a new run, backup all previous test results and clear the "data review" screen.

- 1) Thoroughly mix all calibrators, QC and patient samples before pipetting.

- 2) Prior to loading samples on the instrument, ensure that no air bubbles are present in the sample cups. Break a wood applicator into pieces and use them to pop the bubbles if necessary.
- 3) For a calibration run, use **black** calibrator racks. Open the barcoded calibrators and place them in an unassigned **black** calibrator rack. Nonbarcoded calibrators must be pipetted into sample cups and placed in their assigned positions in **black** calibrator racks. When calibration is completed, the results will be printed.
- 4) To run QC, use the **white** QC racks. If using Roche QC, open the barcoded QC's and place them in the **white** assigned rack and position. For CDC QC, pipette 150 µL of each nonbarcoded QC into a sample cup and place atop the tube in the assigned control position of the **white** QC racks. When the instrument is started, it will automatically run the correct tests on the preprogrammed QC and print the results.
- 5) To run patient samples, use the **gray** sample racks. Place empty sample cups onto barcode labeled 13 X 75 tubes in **gray** sample racks and pipette 150 µL of the serum samples into the sample cup. Pipette 20-25 samples at a time and immediately place the racks on the input buffer tray. **Gray racks with yellow stickers are for urine samples only.** Patient results do not print until requested.

D. Initiating a Run

Do not load samples on the input tray if the green light is flashing.

When the instrument starts, it will run the default profile on all samples unless programmed differently prior to loading.

- 1) Once the calibrator, control or sample racks are loaded on the input tray, they should be measured within 2 hours because of possible evaporation effects. Calibration and QC checks must be completed before pipetting patient samples.
- 2) For detailed, step by step instructions, refer to "**SOP Modular PE Operation**" or the Roche *Modular Analytics[®] Operator's Manual*.

E. Processing and Reporting a Run

- 1) The Hitachi Modular PE Control Module is used to review data and check for samples that need to be diluted or repeated for confirmation. The LIMS database is used for additional levels of data review by the analyst, project lead, QA officer, and supervisor and for data reporting.
- 2) For more detailed information, refer to Section 3 and the "**SOP Computerization and Data System Management**".

F. Special Method Notes

The system can be completely turned off for the weekend or extended holidays or when indicated by maintenance procedure or error code. Refer to the Roche *Modular Analytics[®] Operator's Manual* for instructions.

G. Calculations

All calculations are performed by the Hitachi Mod PE[®] Software system using a machine-stored calibration curve.

H. CDC Modifications

The method is run exactly as stipulated by Roche Diagnostics; CDC has introduced no modifications.

9. Reportable Range of Results and Sample Dilution

The reportable range is defined by the lower detection limit and the maximum of the master curve. For the purposes of CDC reporting, we will use a reportable range of 30-2000 pg/mL. Samples with values of <200 pg/mL will be automatically repeated to confirm the low result. Samples with values below the detection limit are reported as <30 pg/mL. Samples with values of >2000 pg/mL are manually diluted 1:2 with Elecsys Diluent Universal and reanalyzed. Since endogenous Vitamin B12 is present in the Elecsys Diluent, it must be subtracted from the instrument result before the result is multiplied by the dilution factor. The higher the dilution factor, the greater the effect of the endogenous B12.

Note: Sample-dependent non-linearity upon dilution is seen with samples having analyte levels beyond the measuring range. As Elecsys Diluent Universal may contain low levels of endogenous vitamin B12, it is recommended that linearity studies be performed using a known low analyte-containing serum pool. Samples outside the measuring range can be diluted 1:2 with Elecsys Diluent Universal; the effect of endogenous vitamin B12 concentration is insignificant at these levels.

10. Quality Control (QC) Procedures

A. Blind Quality Controls

Blind QC specimens can be inserted into the mix of patient specimens. These QC specimens are generally prepared at two levels that would be encountered in patient samples; the labels used are identical to those used for patient samples. One blind QC specimen randomly selected for concentration is included at a randomly selected location in every 20 specimens analyzed.

B. Bench Quality Controls

Bench QC specimens are prepared from three serum pools, which represent low, medium, and high levels of B12. These pools are prepared in the same manner as patient samples and analyzed in duplicate as part of each run.

Three QC pools per run with two or more QC results (replicates) per pool:

- 1) If all three QC run means are within 2Sm limits and individual results are within 2Si limits, accept the run.
- 2) If 1 of the 3 QC run means is outside a 2Sm limit – reject run if:
 - a. 1_{3s} : Any of the three QC results are outside the 3s limit
 - b. 2_{2s} : Two of the three QC results in the run are outside the 2s limit (same side of mean)
 - c. $10_{\bar{x}}$: Ten sequential QC results (across pools and across runs) are on the same side of the mean.
- 3) If one of the six QC individual results is outside a 2 Si limit – reject run if:
 - a. Outlier – One individual result is beyond the characterization mean ± 4 Si or
 - b. R_{4s} : Sequential QC results (either within the run or across runs) are outside the 2s limit on the opposite sides of the mean

Si = Standard deviation of individual results (the limits are not shown on the chart unless run results are actually single measurements).

Sm = Standard deviation of the run means (the limits are shown on the chart).

Sw = Within-run standard deviation (the limits are not shown on the chart).

The QC results are checked after each run using of a multi-rule quality control program [11]. A QC program written in SAS is available from the DLS Quality Assurance Officer and should be used to apply these rules to QC data and generate Shewhart QC charts. No results for a given analyte are to be reported from an analytical run that has been declared "out of control" for that analyte as assessed by internal (bench) QC. The initial limits are established by analyzing pool material in 20 consecutive runs and then are reevaluated quarterly. When necessary, limits are updated to include more runs. While a study is in progress, QC results are stored in a LIMS database. For runs that are not imported into the database (i.e., R&D, troubleshooting, research-type runs), QC results are stored electronically in the analyte-specific folder on the DLS network. A hardcopy of the QC results from each run is also maintained by the analyst.

11. Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria

- A. Check to make sure that the hardware is functioning properly.
- B. Recalibrate the instrument.
- C. Check reference material.
- D. If the steps outlined above do not result in correction of the "out of control" values for QC materials, consult the supervisor for other appropriate corrective actions.
- E. Call the Roche "hotline" or service engineer.
- F. Do not report analytical results for runs not in statistical control.

12. Limitations of Method; Interfering Substances and Limitation of the Procedures

- A. In patients receiving therapy with high biotin doses (i.e. >5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.
- B. For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.
- C. Assay needs to be performed within 2 hours of the samples being placed on board the instrument to minimize the effect of evaporation.

13. Reference Ranges (Normal Values)

Clinical reference ranges reported in the Roche Vitamin B12 package insert [5] are:

Region	N	Median (pg/mL)	Range (2.5 th -97.5 th percentile) (pg/mL)
USA	178	463	211-946

The newest reference ranges for the U.S. population generated with the Bio-Rad assay for NHANES 2003-2006 are shown below [12]. The Second Nutrition Report also shows reference ranges by population subgroups [12].

Serum vitamin B12: 206-1300 pg/mL (2.5th -97.5th percentile; *n* = 16,316)

14. Critical Call Results (“Panic Values”)

Any sample with B12 levels <200 pg/mL requires follow-up (repeat analysis for confirmation of low B12 level). Since survey data are transmitted several times weekly to Westat, abnormal reports are automatically forwarded to the NCHS survey physician for follow-up. For smaller, non-NHANES studies, abnormal values are identified to the study principal investigator. Emails sent concerning abnormal results are maintained by the supervisor for the duration of the study. Most of these studies are epidemiological in nature.

15. Specimen Storage and Handling during Testing

Specimens are allowed to reach room temperature during preparation. The unused portion of the patient specimen is returned to the freezer.

16. Alternate Methods for Performing Test; Storing Specimens if Test System Fails

If the analytical system fails, we recommend that the specimens be stored at $\leq -20^{\circ}\text{C}$ until the analytical system is restored to functionality.

17. Test Result Reporting System; Protocol for Reporting Critical Calls (If Applicable)

Test results that are not abnormal are reported to the collaborating agency at a frequency and by a method determined by the study coordinator. Generally, data from this analysis are compiled with results from other analyses and sent to the responsible person at the collaborating agency as an ASCII text file or Excel file, generally through electronic mail or via ftp site.

For NHANES 1999+, all data are reported electronically weekly to Westat who then transfer the results to NCHS. For some smaller studies, hard copies of a data report are sent, as well as the results in electronic format.

18. Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking

The LIMS database is used to keep records and track specimens for NHANES 1999+. If analyses are performed for smaller, non-NHANES studies, records may be kept in Excel files on the DLS network.

We recommend that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study. Only numerical identifiers should be used (e.g., case ID numbers). All personal identifiers should be available only to the medical supervisor or project coordinator. Residual serum from these analyses for non-NHANES studies may be discarded at the request of the principal investigator, or may be transferred to the CDC CASPIR facility for use by other investigators. Very little residual material will be available after NHANES analyses are completed, and these vials may be routinely autoclaved.

The exact procedure used to track specimens varies with each study and is specified in the study protocol or the interagency agreement for the study. Copies of these documents are kept by the supervisor. In general, when specimens are received, the specimen ID number is entered into a database and the specimens stored in a freezer at -70°C . The specimen ID is read off of the vial by a barcode reader attached to the computer used to prepare the electronic specimen table for the analytical system. When the analyses are completed, the DIF file containing the electronic copy of the results is loaded into the

database, and the analytical results are linked to the database by ID number. The analyst is responsible for keeping a notebook containing the ID numbers of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies.

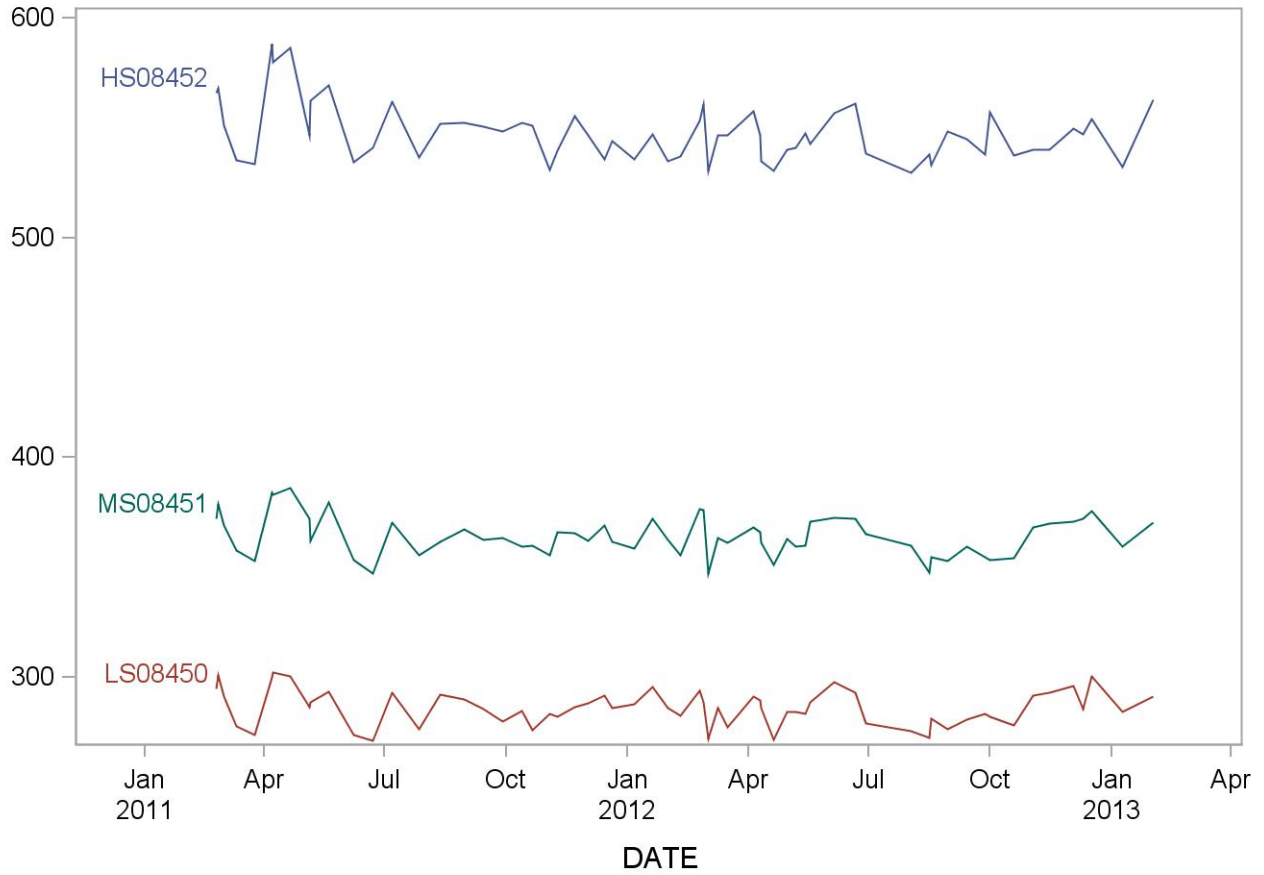
19. Summary Statistics and QC Graphs

See following pages.

Summary Statistics for Vit. B12 (pg/dL)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HS08452	71	24FEB11	01FEB13	547.4	13.2	2.4
LS08450	71	24FEB11	01FEB13	286.1	8.3	2.9
MS08451	71	24FEB11	01FEB13	364.2	9.4	2.6

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References

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- (10) Roche Modular Analytics Operator's Manual.
- (11) Caudill SP, Schleicher RL, Pirkle JL. 2008. Multi-rule quality control for the age-related eye disease study. *Stat Med* 27:4094-4106.
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ACKNOWLEDGMENTS

We gratefully acknowledge the contributions of Donna LaVoie and Christine Pfeiffer who assisted in evaluating this assay and preparing the manuscript for this chapter.

Appendix 1 – Method Figures of Merit

Accuracy:

NIBSC International Standard 03/178 (reconstituted and diluted with water):

The Roche E-170 vitamin B12 assay shows a small positive bias (<7%) relative to the assigned consensus mean (480 pg/mL) at low dilution (up to 1:2). At higher dilution the assay over-recovers due to the lack of protein matrix.

NIBSC International Standard 03/178 (reconstituted with protein diluent and spiked into serum):

The Roche E-170 vitamin B12 assay shows complete recovery (within $\pm 10\%$) if spiking level is similar to endogenous level. If the spiking level is low (20%) compared to the endogenous level, the recovery is low (77%).

Precision:

The within-day imprecision ($n = 10$ replicates) of the Roche E-170 vitamin B12 assay is <2.1%.

The between-day imprecision ($n = 30$ days) is <5.5% at concentrations of 288-545 pg/mL.

Dilution linearity:

A serum sample with a high vitamin B12 concentration (1644 pg/mL) was diluted with the Roche Diluent Universal up to 1:64. If the B12 background contribution of the diluent was considered (subtracted), good dilution linearity ($r^2 = 0.996$) was obtained and the measured concentrations corresponded well (within $\pm 10\%$) with the expected concentrations.

Limit of detection (LOD):

The manufacturer specification for LOD is 30 pg/mL.

In-house determined LOD: 11 pg/mL (BioRad protein diluent); 40 pg/mL (Roche Diluent Universal)

Determination of the LOD by serially diluting the "low" QC pool with water and estimating the SD at a concentration of zero (σ_0) by extrapolating repeat analyte measurements ($n = 9$) made near the detection limit in these dilutions (LOD defined as $3 \sigma_0$).

Both of the in-house determined LOD values were similar to the manufacturer specified LOD. The manufacturer specified reportable range starts at 30 pg/mL.

Vitamin B12 levels <100 pg/mL are extremely rare in the general US population in NHANES (<0.2%).