

Laboratory Procedure Manual

Analyte: *Cockroach (Bla g 1)*

Matrix: **Dust**

Method: **Enzyme-linked immunosorbent assay
(ELISA)**

Method No.:

Revised:

as performed by: *Air Quality Sciences
Atlanta, Ga*

Contact: *Dr. Elliott Horner*

Important Information for Users

Air Quality Sciences 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 NHANES 2005–2006 data.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label
ALDUST_D	DSXBG1	Bla g 1 Result (U/mL)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

This method describes the procedure for determining specific allergens in samples, using commercially available enzyme-linked immunosorbent assay (ELISA) test kits from Indoor Biotechnologies (InBio) Inc. (Charlottesville, VA). ELISA testing provides a quantitative analysis for a specific allergen. The allergen is measured using monoclonal capture and polyclonal detection antibodies, a process of antibody / antigen recognition. The reaction from an enzyme conjugated to the detection antibody generates a colored product; the intensity of the color is proportional to the concentration of the allergen. The concentration is determined by measuring color intensity at a specific wavelength.

The literature and instructions with each kit constitute the standard operating procedure for the method. The test is to be conducted by qualified analysts who have received training on dust allergen analysis by ELISA assay.

Bla g 1 is an allergen from the German and the American cockroach, which is typically found in the kitchen and bathroom. The clinical relevance is that exposure to higher reservoir concentrations can increase the risk of sensitization and may precipitate asthma symptoms. From a clinical perspective, the data can be used to either validate or establish the allergen levels associated with an increased risk of sensitization. From an individual's perspective, the data can be used to determine if action is needed to reduce exposure and perhaps subsequently reduce risk to health. It can also verify allergen reduction efforts.

2. SAFETY PRECAUTIONS

- A. Material Safety Data Sheets (MSDS) for all chemicals and reagents used in the microbiology laboratory are available to all laboratory personnel.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

- A. The AQS Data Manager (ADM) system, developed by Air Quality Sciences, Inc., is used to record each set of samples with a separate Project/Product identifier for tracking, reporting, and billing purposes. All files stored on ADM are automatically backed up nightly to tape. The tapes are kept onsite in a fireproof safe, and monthly moved to offsite storage. Hard copy documentation is stored for three years.
- B. Sample numbers are assigned individually to samples within each set of samples. Individual samples received from CDC will be checked in under the appropriate Project/Product and tracked using an internal barcode tracking system from check-in to disposal or storage into a cryo tube for return shipment to CDC. The internal AQS barcode number is used to determine the location of the sample at any time and the well locations in microtiter plates used for testing each sample.
- C. Calculations of allergen concentrations are computer generated by the plate reading software. The software compares test values with the linear portion of a control curve. Samples with test values below the low cutoff value are reported as below limit of detection, while samples with test values above the high cutoff value are retested with increased dilution. The test values are reviewed by a second qualified individual other than the analyst who performed the test to determine acceptability of the results based on statistical analysis. The accepted test values are then electronically transferred to ADM where they are automatically formatted to the prescribed reporting scheme. Final reports are emailed to the WESTAT upon the completion of Bla g 1 tests for each sample batch.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

- A. Sample extracts are received by lab services, assigned a unique AQS barcode identifier, and delivered to the microbiology laboratory for analysis.
- B. Any samples received with packaging broken or contents spilled are rejected and the CDC will be notified in the final report.
- C. Sample extracts are stored at -20°C.
- D. If less than 60 µl of eluate sample (20 µl for Bla g 1 and 25 ul for Aspergillus fumigatus is received, it may not be possible to run both the assays (Bla g 1 and Aspergillus fumigatus. Assay analyses are to be conducted in the priority specified by the CDC (Bla g 1 and then Aspergillus fumigatus).
- E. All antibodies and standards are stored according to manufacturer's instructions.
- F. Date of receipt of antibodies, standards, and kits is recorded on labels upon arrival.
- G. Date opened is recorded on label of ELISA antibodies and standards when use begins.
- H. All solutions and reagents are stored according to manufacturer's instructions.
- I. Any samples received in poor condition are reported back to the CDC in the deliverable report.

5. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

- A. The following instrumentation is employed to analyze dust allergens at AQS:
 1. SpectraMax 340pc (Molecular Devices, Sunnyvale, CA)
 2. SpectraMax Pro software Version 5.0.1 (Molecular Devices, Sunnyvale, CA).
 3. Analytical balance capable of weighing up to 10 g and as low as 0.0001 g (Precisa Balances, Switzerland, Precisa Type 300-9204)
 4. Oakton pH/Ion 510 Bench pH meter (Oakton Instruments, Vernon Hills IL)
- B. The following materials are employed to analyze dust allergens at AQS:
 1. Eppendorf Research 10 µl single channel micropipettor (Fisher Scientific, Suwanee, GA, Cat. No. 05-402-88)
 2. Eppendorf Research Pro 100 µL single channel micropipettor (Fisher Scientific, Suwanee, GA, Cat. No. 22-46 131-1)
 3. Eppendorf 1000 µl single channel micropipettor (Fisher Scientific, Suwanee, GA, Cat. No. 05-402-90)
 4. Eppendorf Research Pro 300 µL multi-channel micropipettor (Fisher Scientific, Suwanee, GA, Cat. No. 22-46 142-7)
 5. Eppendorf 200 µL pipette tips (Fisher Scientific, Suwanee, GA, Cat. No. 05-403-66)
 6. 10 mL serological pipettes (Fisher Scientific, Suwanee, GA, Cat. No. 13-676-10J)
 7. Nunc 96 well microplates (Fisher Scientific, Suwanee, GA, Cat. No. 125-65-135)
 8. 15 mL plastic screw cap graduated tubes (Fisher Scientific, Suwanee, GA, Cat. No. 05-539-5)
 9. 50 mL plastic screw cap graduated tubes (Fisher Scientific, Suwanee, GA, Cat. No. 05-539-6)
 10. Traceable timer(s) (Fisher Scientific, Suwanee, GA, Cat. No. 06-662-5)
 11. Solution basins (Fisher Scientific, Suwanee, GA, Cat. No. 13-681-100)
 12. Versi-Dry lab soakers (Fisher Scientific, Suwanee, GA, Cat. No. 12-007-186)
 13. Plate Washer machine (Molecular Devices)
 14. Kimwipes (Fisher Scientific, Suwanee, GA, 06-666A)
- C. Reagent preparation
 1. All antibodies and standards for Bla g 1 are manufactured by Indoor Biotechnologies, Inc.
 2. All solutions and reagents are prepared according to manufacturer's instructions.
 3. Recipes for all ELISA solutions and reagents are located in the Microbiology Laboratory's Media book.

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4. Lot numbers are assigned and the lot numbers of chemicals used in production of reagents are designated via the worksheet found in the ELISA Notebook.

70% ISOPROPANOL (Labchem Inc.—flammable liquid, causes irritation to eyes, skin, mucous membrane)

50mM CARBONATE-BICARBONATE BUFFER, pH 9.6 +/- 0.2

Na ₂ CO ₃	1.59 g
NaHCO ₃	2.93 g
Thimerosal (Optional)	0.1mL
Distilled Water	1000 mL

PHOPHATE BUFFERED SALINE (PBS), pH 7.4 +/- 0.2

NaCl	8 g
KH ₂ PO ₄ , Monobasic	0.2 g
Na ₂ HPO ₄	1.15 g
KCl	0.2 g
Thimerosal (Optional)	0.1 mL
Distilled Water	1000 mL

PHOSPHATE BUFFERED SALINE WITH 0.05% TWEEN 20, pH 7.4 +/- 0.2 (PBS-T)

PBS	999 mL
Tween 20 (50%)	1 mL

1% BOVINE SERUM ALBUMIN IN PHOSPHATE BUFFERED SALINE WITH 0.05% TWEEN 20 (PBS-T + BSA)

pH 7.4 +/- 0.2

PBS-T	500 mL
BSA (Bovine Serum Albumin)	5 g

ABTS IN CITRATE-PHOSPHATE BUFFER, pH 4.2 +/- 0.2

Phosphate-Citrate Buffer Tablets	5 tablets
Distilled Water	500 mL
ABTS (Azino -bis (3-ethylbenzothiazoline 6-sulfonic acid) Triammonium Salt	0.274 g

Prepare Phosphate-Citrate Buffer first then add ABTS. ABTS is light sensitive.

GOAT ANTI-RABBIT HRP CONJUGATE (Millipore cat# 12 348)

D. Bench control samples

1. Bench control samples are supplied by indoor biotechnology. The "bench control" samples are stored at -20°C.

E. Preparation of quality control material

1. Two "bench control" samples are included in each microplate. The bench control samples are targeted for 1) midrange-to-bottom, and 2) midrange-to-top of the linear portion of the standard curve.
2. The positions of the bench control samples on the microplate are picked from random number generator.
3. Since bench control samples are used neat and not diluted only three wells per plate are used for low and high bench control and middle well is left blank.
4. Values are plotted on a control chart and evaluated for acceptability.

6. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

- A. Internal calibration curves are included in the analysis of each batch (plate).
 1. Purified standards are used as provided by the manufacturer.
 2. Ten serial two-fold dilutions of the standard are prepared in duplicate on the plate.
 3. Concentrations range as follows: 1.000 – 0.002 U/ml.
 4. Absorbance values of the calibration wells are regressed on the concentration using the 4-parameter curve fit routine in SoftMax pro software.
 5. Calibration curves are adjusted as described below.

7. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

Procedure

A. Coating of Microplates

1. Label each microplate with the barcode from ADM containing the plate name, date, and allergen type.
2. Prepare capture antibody solution by adding InBio capture antibody anti Bla g 1 mAb MA-10A6 to carbonate-bicarbonate solution at a 1:1000 concentration (10 μ L per 10 mL).
3. Dispense 100 μ L of capture antibody solution to all wells.
4. Incubate overnight at 4°C.
5. Coated microplates can be used if incubated at 4°C. Incubation of coated microplates must not exceed 72 hours.
6. Record the reagent lot numbers on the ELISA worksheet.

B. Wash

1. Remove coated microplate(s) from 4°C storage.
2. Wash all wells 3x with 300 μ l of PBS-T.

C. Addition of Blocking Agent

1. Dispense 200 μ L of 1%BSA PBS-T (see 5.C) to each well.
2. Incubate for thirty minutes at room temperature.
3. Blocked microplate(s) can be stored overnight at 4°C if process must be stopped in emergencies.
4. Record the reagent lot numbers on the ELISA worksheet.

D. Wash

1. Wash all wells 3x with 300 μ l of PBS-T.

E. Loading and Dilution of Standard and blank

1. The standard curve uses twenty wells on the microplate. The standard is run in duplicate and diluted by ten, serial two-fold dilutions.
2. Add 20 μ L of InBio Bla g 1 (ST-BG1) allergen standard to 180 μ l PBS-T in wells A1 and B1. Mix thoroughly before transfer.
3. Transfer 100 μ L from wells A1 and B1 to wells A2 and B2, respectively.
4. Continue transfer and suspension of 100 μ L through wells A10 and B10. Mix the contents of the well thoroughly prior to each transfer.
5. Discard 100 μ L from wells A10 and B10.
6. Load only the diluent (1%BSA-PBS-T) in the blank wells.
7. Incubate microplate(s) for one hour at room temperature
8. Record the reagent lot numbers on the ELISA worksheet.

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F. Sample and bench control Loading and Dilution

1. Dispense 100 μ L of PBS-T + BSA (see 5.C) to all wells except the wells assigned for bench controls.
2. Add 80 μ L PBS-T + BSA to wells A1-H1, C4-H4, C7-H7, C10-H10.
3. For microplates that will have dilutions beyond the usual three, add 80 μ L PBS-T+BSA only to wells that will receive sample directly as designated by the analyst.
4. Check ADM NHANES test barcode report to determine which samples are to be tested next (no test date entry next to test name) and/or repeated (previous test date in bold). Add 20 μ L of eluate from each sample individually to a sample well (C1-H1, C4-H4, C7-H7, C10-H10). Mix thoroughly before transfer.
5. Verify that wells for bench controls are empty. Load bench controls samples by dispensing 100 μ L of appropriate solution to well(s) identified randomly. Vortex thoroughly before dispensing.
6. Transfer 100 μ L of diluted sample eluate to the next well horizontally to the right (e.g. C1 to C2) and repeat to the next well (e.g. C2 to C3). Mix thoroughly prior to each transfer. Discard 100 μ L from the second dilution well. Repeat for the other sample wells. Initial dilutions are 1:10, 1:20, and 1:40.
7. Incubate microplate(s) for one hour at room temperature.

G. Wash

1. Wash all wells 3x with 300 μ l of PBS-T.

H. Load 2nd Antibody

1. Prepare second antibody (InBio PA-BG1 Rabbit anti-Bla g 1) solution by diluting with 1% BSA-PBS-T (see 5.C) at a 1:1000 concentration. (10 μ L per 10 mL)
2. Dispense 100 μ L of solution to all wells.
3. Incubate microplate(s) for one hour at room temperature.
4. Record the reagent lot numbers on the ELISA worksheet.

I. Wash

1. Wash all wells 3x with 300 μ l of PBS-T.

J. Loading Conjugate

1. Prepare conjugate solution by adding Peroxidase Goat anti-rabbit IgG to 1%BSA- PBS-T (see 5.C) at a 1:1000 concentration (10 μ L per 10 mL).
2. Dispense 100 μ L of conjugate solution to all wells. See above
3. Incubate microplate(s) for one hour at room temperature.
4. Record the reagent lot numbers on the ELISA worksheet.

K. Wash

1. Wash all wells 3x with 300 μ l of PBS-T.

L. Addition of Substrate

1. Prepare substrate solution by adding 30% Hydrogen Peroxide to ABTS at a 1:1000 concentration (10 μ L per 10 mL).

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2. Add 100 μ L of ABTS/Peroxide solution to all wells immediately after mixing. Solution must be used within an hour after mixing with H_2O_2 . We use this final solution within about an hour, as it goes green soon.
3. Record the reagent lot numbers on the ELISA worksheet.
4. Observe color change (from clear to green). Approximate development time is 5-15 minutes.
5. Insert microplate into plate reader and observe the OD readings for the two lowest dilutions (wells A1 and B1), and record when the OD reading for both wells are between 2.0 and 2.4.

M. Reading of Plates

1. The SPECTRAmax 340PC microplate spectrophotometer provides rapid and sensitive measurements of a variety of analytes across a wide range of concentrations. It measures the optical density (OD) of samples in a microplate at a selected wavelength for a single point in time.
2. Turn on SpectraMax 340pc using the switch located on the back panel, and access the SoftMax Pro software on the computer connected to the plate reader.
3. Set the SpectraMax 340pc for an Endpoint reading with 405nm and the temperature to 24.3°C. Adjust the wavelength by pressing the up or down arrow keys above and below the λ to increase or decrease the wavelength setting. Adjust the temperature by pressing the up or down arrow keys above and below the TEMP to increase or decrease the temperature setting.
4. In SoftMax Pro, open the ELISA folder of the current year (found in G:/MICROLAB/PLATE READER/).
5. Open the appropriate allergen folder of the current year.
6. Open the allergen template.
7. Save the template with the plate name/date/test type by reading the plate barcode as new filename.
8. Wipe the bottom of the ELISA plate with a Kimwipe dampened with 70% isopropanol to remove dust, fingerprints etc.
9. Place the plate in the SpectraMax 340pc drawer with well A1 of the plate going to the top left-hand side of the tray.
10. Click the "read" button in SoftMax Pro to read the plate.
11. When the OD reading for the standards in well A1 and B1 are between 2.0 and 2.4, save the data in the filename from M.7.
12. Save the file in its appropriate folder.
13. New ELISA and allergen folders are to be created at the beginning of the year, or more frequently as appropriate.

N. Adjusting the Standard Curve in SoftMax Pro

1. In SoftMax Pro, remove (mask) standard points/data by highlighting desired standard well(s) on the template.
2. Points are taken out (i.e. masked) from the non-linear portions of the curve (top and /or bottom) if necessary to obtain an R^2 of 0.995 or better for the standard curve.
3. Points are taken out that will leave standard CV% values of 20.0 or under.
4. Standard curves must contain at least five dilution points. Masking of adjacent points is permitted only from either end of the curve.
5. The working Lower Limit of Detection (LLOD) must be ≤ 0.40 U Bla g 1 allergen/ml.
6. Print out the edited datasheet in portrait form and attach to ELISA worksheet.

O. Analyzing the Datasheet From SoftMax Pro

1. Standards and Plate Settings
 - a. If the reading was not Endpoint and the wavelength not 405nm, the plate must be discarded and repeated to be read at the proper settings.

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- b. On the SoftMax Pro datasheet check for the setting of Endpoint and make sure the reading was at 405nm in the box beside the template that describes the settings of the SpectraMax 340pc.
- c. Check the CV% values of the standards and underline any that are over 20.
- d. Note and box the smallest standard value and largest standard value comparing to the largest and smallest standard Mean Values. These values indicate the range of the standard curve for this particular plate.
- e. Note and circle the standard curve R^2 value.
- f. Circle the BackCalcConc of the most diluted (unmasked) standard.

2. Unknowns' Data

- a. Each sample dilution result is compared to the linear portion of the standard curve. Those results not located on the linear portion of the standard curve are rejected (assigned an "R" for out of range) and not reported.
- b. The result(s) not rejected are evaluated for reporting. The values from all dilutions for a test specimen that are within range are averaged and reported.
- c. If all sample dilution results are rejected, AQS software determines if the sample is above or below the standard curve range by comparing the value of the sample to the largest and smallest unmasked standard values (endpoints of the linear portion of the standard curve).
 - i. If all values for a sample are assigned an "R" to indicate that the sample is below the lowest value of the linear portion of the standard curve, then the measured value and the LLOD (lower limit of detection) are reported and annotated with code "37".
 - ii. If the R's indicate that the amount of allergen in the sample is above quantifiable levels for the run (the calculated concentration is higher than the highest value of the linear portion of the standard curve), the results of one additional set of higher dilutions are evaluated on a subsequent run. The highest dilution from the initial run is to be the starting dilution on the follow-up test.
 - iii. If all values for a sample in the second set of dilutions are assigned an "R" to indicate that the sample is above the highest value of the linear portion of the standard curve, then the value for the highest concentration of the standard is reported and annotated with code "102".
- d. Staple the ELISA Worksheet created for the allergen to the front of the SoftMax Pro datasheet for each plate ran that day. A second qualified person is to review the worksheet.
- e. If the plate will not be used record the reasoning on the ELISA Worksheet.

P. Transferring Data from SoftMax Pro to Report Spreadsheet

Upon completion of each allergen test plate read, the data is saved as a SoftMax Pro data file and exported to the network as a text file to be used by ADM to update the test specimen data spreadsheet supplied by WESTAT. A barcode system used to assign each eluate sample with a unique AQS ID upon arrival links the analyte results to the appropriate CDC ID in the e-file formatted for reporting. An automated routine will examine the designated network directory for the presence of a new allergen test plate file, and when found, it will extract the information into the e-file. The AQS barcode number is recorded as the run number in the e-file. The routine determines whether the information being retrieved is accepted as a result. This is done by checking whether the result was accepted or had an "R" for range. The values from all dilutions for a test specimen that are within range are averaged together. This averaged value is reported as the final result in the test specimen data spreadsheet. If no samples are accepted, the routine determines whether the cause is due to all values being less than the LLOD. If the reason is all values less than the LLOD, the LLOD value and comment code 37 is recorded.

Q. Calculations

1. Calculations are computerized within SoftMax Pro.
2. Bla g 1 allergen is reported as U/ml (Units of allergen per ml of sample).
3. To obtain the allergen concentration, calculations are completed through SoftMax Pro as follows:
 - a) Multiply the adjusted result by the dilution factor.
 - b) Round the result to two decimal points.

Quality Control Materials

Two Bench Control samples prepared by Indoor Biotechnologies at different points on the standard curve are included as samples in each run.

Operation

Two bench control samples are included in each plate run. Reported values are compared to values for bench control samples from prior runs.

CDC Modifications

None.

Special Method Notes

None.

8. REPORTABLE RANGE OF RESULTS:

The range of detection with this method for the first round of dilutions (nominal 1:10, 1:20, 1:40) is from 0.002 to 1 U Bla g 1 allergen/ml of eluate. Higher concentrations are determined through additional dilutions of the sample eluate and adjustment of the accepted result by the dilution factor. For informational purposes, these allergen (in eluate) concentrations correspond nominally to 0.4 to 20 U Bla g 1 allergen/g dust at the recommended elution ratio. Due to the intrinsic variability from plate to plate, a lower limit of detection is determined individually for each plate. Typically, values less than 0.002 U/ml are reported and flagged with comment code "37" and the LLOD for the plate is reported. Values greater than 1 U/ml are verified by re-assay with further dilution (1:40, 1:80, 1:160). Values greater than the upper limit of quantitation for the 2nd assay are reported with comment code "102" along with the upper limit of quantitation for the plate.

9. QUALITY CONTROL (QC) PROCEDURES

In order to ensure that analytical data are accurate, precise, and generated from acceptable procedures, the following quality control procedures have been enacted:

- Comparison studies are performed between different reagent lots;
- Bench control samples are randomly placed in two sample positions on each plate;
- Each plate contains replicate dilutions for the standard curve and two bench control samples;
- Shewhart rules using control chart are used for monitoring the following QC results for each plate run: bench control concentrations (U/ml) and the Lower Limit of Detection (LLOD) for the bottom of the standard curve. Each run will be evaluated against the control charts. The "system" (i.e. testing process) is declared "in control" if QC results are within the limits listed below:
 - a. Bench Controls: both high and low controls must fall within $\pm 2s$ of the fixed mean value determined from interplate and intraplate precision studies conducted with the bench controls, respectively;
 - b. Criteria for acceptability of standard curves must be met as described in Section 7.N.

- If a run is not accepted or fails any of the statistical tests (see below), the run will be repeated. The following statistical rules are applied to each run for acceptability:
 - a. Any QC result outside the acceptable limits fails the plate.
 - b. Four of five sequential results of either the high or low bench control between 1s and 2s on the same side of the mean.
- A QC program will be used to apply these rules to the QC data and generate QC charts. Fixed, rather than “trailing average”, values will be used for calculating the mean and standard deviation. The mean and standard deviation are established by analyzing material in 20 or more consecutive runs from the pilot study. No results for a given analyte are to be reported from an analytical run that has been declared “out of control”. Electronic copies of the QC results and of the tracking of the QC results over time from each run are stored on the network. The analyst also keeps a hardcopy of the QC results from each run.

10. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

Quality control charts will allow visual analysis of system performance and observation of anomalous or unacceptable deviations. The need for corrective action may be identified through reviews, internal QC checks, audits or observations made during routine sampling and analysis activities by project staff. All corrective actions will be documented. No further work may be performed until the problem has been satisfactorily resolved, and the Quality Manager has acknowledged approval. QA checks of data as early as possible are essential to provide early warning of potential problems.

11. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

- A. The work area is cleaned before starting the each assay.
- B. Microplates are covered during incubation periods to prevent dust or particulate matter from settling into wells and compromising assay results.
- C. ABTS is light sensitive and may degrade if exposed to light sources. Aluminum foil is placed around all bottles containing ABTS to inhibit penetration of light into ABTS solution.
- D. Fingerprints or other markings from lab bench and area may interfere with reading of microplates. The bottom and sides of each plate is wiped down with a Kimwipe and 70% Isopropanol immediately before plate is placed into spectrophotometer.
- E. Plates may not develop properly if the pH of ELISA reagents is incorrect. If this is suspected, the pH is taken and the lot discarded if pH is out of range. Fresh reagents are then produced and the plate repeated.
- F. Plates may not develop properly due to old or improperly mixed ABTS. The plates must then be repeated with freshly made ABTS and the old lot discarded.
- G. If solution becomes contaminated the lot is discarded and the solution remixed

12. REFERENCE RANGES (NORMAL VALUES)

There are no established ranges for this type of testing. The allergen level associated with an increased risk of sensitization among atopic individuals is 1 Unit allergen per gram of dust.

13. CRITICAL CALL RESULTS (“PANIC VALUES”)

AQS will notify WESTAT of results through electronic transfer. For NHANES 2010, WESTAT automatically notifies the survey physician because of the weekly electronic transmission of data.

14. SPECIMEN STORAGE AND HANDLING DURING TESTING

Test Specimens are stored at -20°C in a non frost free freezer.

15. ALTERNATIVE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

There are no acceptable alternative methods for performing this test. In case of system failure, store all test specimens at -20°C until the system is functioning.

16. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

WESTAT will be immediately notified via e-mail. The NHANES Medical Officer will then notify the study participant.

17. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

None.

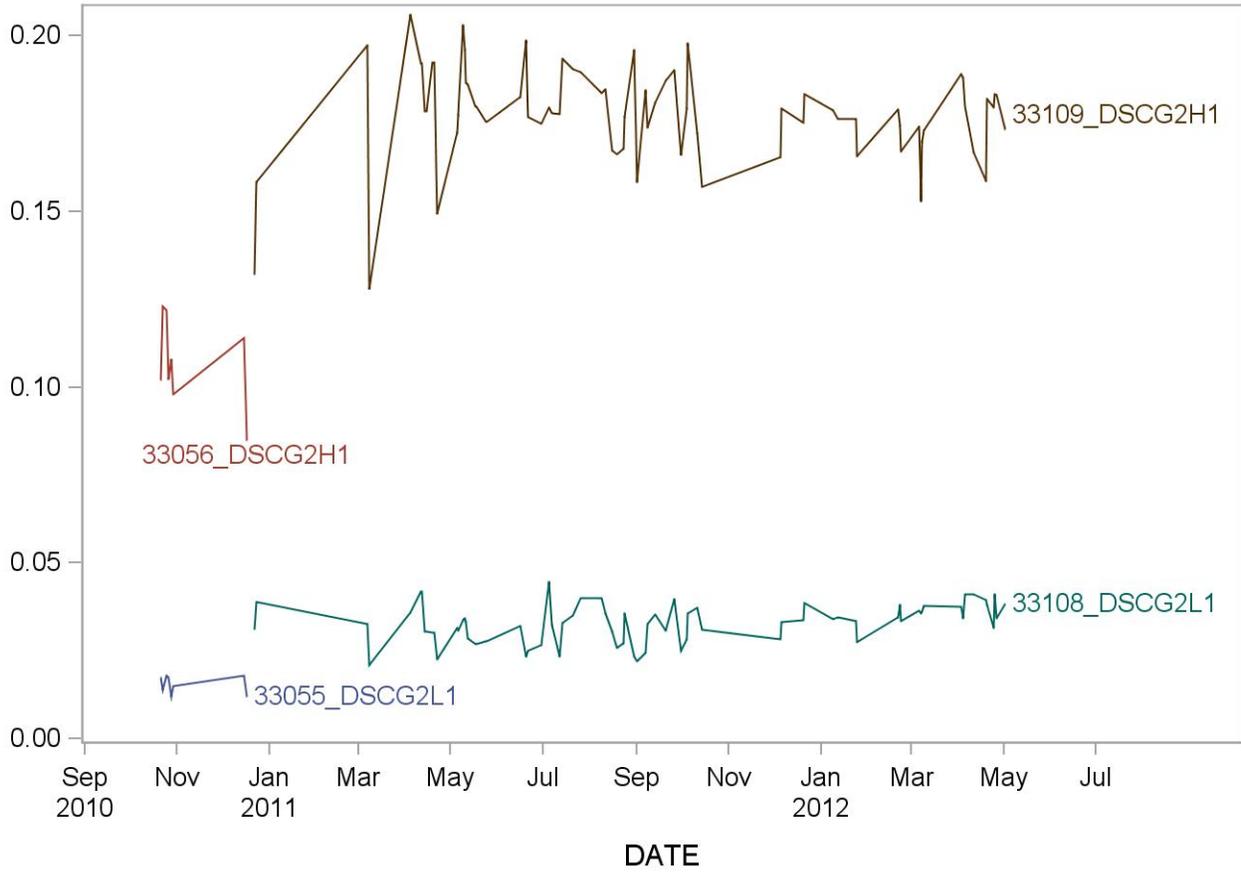
18. SUMMARY STATISTICS AND QC GRAPHS

See following page.

Summary Statistics for German Cockroach (Bla g 1) (U/mL)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
33056_DSCG2H1	15	21OCT10	17DEC10	0.1029	0.0149	14.5
33055_DSCG2L1	15	21OCT10	17DEC10	0.0157	0.0025	16.0
33109_DSCG2H1	319	22DEC10	02MAY12	0.1783	0.0201	11.2
33108_DSCG2L1	319	22DEC10	02MAY12	0.0336	0.0065	19.4

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REFERENCES

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- NHANES Spreadsheet Final QC and Results Transmission MLI150.