

# Laboratory Procedure Manual

*Analyte:*        *Endotoxin*

*Matrix:*        **Dust**

*Method:*        ***Limulus amebocyte lysate assay***

*Method No.:*

*Revised:*

*as performed by:*        *University of Iowa  
Iowa City, Iowa*

*Contact:*        *Dr. Peter Thorne*

## **Important Information for Users**

University of Iowa 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:

<b>Lab Number</b>	<b>Analyte</b>	<b>SAS Label</b>
ALDUST_D	DSXENX	Entotoxin Result (EU/mL)

## **1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE**

Dust endotoxin was also measured with the dust allergens. Endotoxin is an inflammatory lipopolysaccharide from gram-negative bacteria that is found in the indoor environment. Indoor sources of endotoxin include pets, pests, humidifiers and outdoor air. The inhalation of dust endotoxin has been linked to asthma, allergic rhinitis and wheezing.

The dust endotoxin is measured using a Limulus ameobocyte lysate (LAL) assay<sup>3</sup>. The LAL assay is based on the sensitivity of an enzymatic clotting cascade in the ameobocytes found in the hemolymph of the horse-shoe crab *Limulus polyphemus*. The dust extracts were measured using a kinetic chromogenic assay. Endotoxin from *E. coli* 0155 was used in standard curves. Aliquots of endotoxin standards and extract were pipetted into a pyrogen free microplate and LAL reagent and substrate was added. The absorbance was read at 405 nm.

## **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.
- B. Dust is to be handled in a biosafety hood during sieving and weighing. Laboratory personnel are to wear lab coats and latex gloves.
- C. Work areas are washed down with 70% Isopropanol before and after each assay.
- D. All materials to be discarded are to be placed in biosafety bags, autoclaved, and disposed of in normal waste stream.

## **3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT**

- A. The Data Manager system 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 each sample within a set of samples. Individual samples received from CDC will be checked in and tracked using an internal barcode tracking system from check-in to disposal or storage.

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

- Samples are shipped by the NHANES contractor on dry ice in 2 ml pyrogen-free, polypropylene vials with screw caps (Nalgene cryovials) containing from 0 to 100.00 mg sieved reservoir dust from NHANES households (NHANES dust). All samples are stored at -80°C in plastic or fiberboard storage boxes containing up to 81 samples/box. Each aliquot of dust is labeled with an

identification number and the date. To avoid misinterpretation of handwritten numbers, all labels are typewritten and bar coded.

- Each shipment contains a hard-copy manifest that must correctly identify each sample in the shipment.
- An electronic copy of the sample manifest is sent via email to Dr. Nervana Metwali before the shipment is received.
- Upon receipt, samples are inventoried and checked against the manifest by laboratory personnel.
- The sample IDs, weights, and date of receipt are recorded in a custody sheet created by the PTF project staff. Missing, damaged, or additional samples are also noted.
- Discrepancies between the inventory and the manifest are reported to NHANES. Details regarding missing, damaged, or additional samples are addressed via e-mail at that time.
- After inventory, the samples are stored in their original, labeled boxes at -80°C on a designated shelf in the NHANES freezer located in room #194 of the Institute for Rural and Environmental Health (IREH). This freezer is on the PTF alarm system and has a liquid nitrogen backup tank attached.
- The NHANES dust samples are stored at -80°C until they are weighed, extracted and assayed for endotoxin content.

**5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES**

A. Not applicable for this procedure.

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

Supplies provided by NHANES:

- 1) Pyrogen-free, polypropylene vials with screw cap for aliquoting samples for endotoxin and allergen assays (Nalgene Cryovials, cat# 5000-0020 are used in in Phase I and Sarstedt Microfuge cat# 72.694.006 are used in Phases II and III).
- 2) Bar code labels with sample ID#

Supplies purchased from Lonza, Inc.:

- 1) Limulus Amebocyte Lysate (lot# 0000170430)
- 2) Pyrogen-free LAL water (pfw) (lot# 0000173961)
- 3) Control standard endotoxin from *E. coli* 0155 (lot# 0000135999)

Supplies purchased from Fisher Scientific and Rainin:

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- 1) Pyrogen-free, polypropylene vials with screw cap (SARSTEDT, cat# 2013-02) (These vials are used in weighing NHANES dust samples)
- 2) Culture Borosilicate Tubes, cat# 14-961-26
- 3) CMFRT low endotoxin exam gloves (M) cat# 19-167-030C
- 4) CMFRT low endotoxin exam gloves (L) cat# 19-167-030D
- 5) Kimwipes Disp cat#06-666-1A
- 6) EPTPS, RCK, 2.5ml, cat# 05-403-13
- 7) Cent tube 50ml, cat#05-539-6
- 8) Pipette STR 50ml, cat# 13-676-10R
- 9) Pipette STR 25ml, cat# 13-676-10K
- 10) 96 well plate, Costar cat# 07-200-89
- 11) Pipette Basin STRL PS 50ml, cat# 13-681-501
- 12) Laser Tough Tag white, cat# NC9006563
- 13) Alcohol reagent 4L, cat# A962P-4
- 14) Tween-20, cat#BP337-500
- 15) PBS Phosphate Buffer Saline, cat# BP3991
- 16) Rainin tips (pyrogen free tips): 250µl (GPS), 500µl (GPS), 20µl (LGPS)

Pipettors:

- 1) Drummond pipetaid (Fisher Scientific)
- 2) Multichannel pipettor (Pipetman, Gilson)
- 3) 250µl; 1000µl single pipettor ((pipetman, Gilson)
- 4) 10 µl (LTS pipette, Rainin)

\*All pipettors are calibrated at the start of the study and at least annually thereafter

Instruments:

- 1) MT-5 Microbalance (Mettler-Toledo, Inc.)
- 2) XP26 Microbalance (Mettler-Toledo, Inc.)
- 3) Microplate reader (M5, Molecular Devices, Inc.)
- 4) Refrigerated centrifuge (5810R, Eppendorf, Inc.)
- 5) Laminar flow hood (Biological Safety Cabinet Class II A/B3, NuAire, calibrated and certified annually)

Software:

- 1) BalanceLink (Mettler-Toledo, Inc.)
- 2) Office Suite 2010 including Excel, Word, PowerPoint, and Outlook (Microsoft, Inc.)
- 3) Softmax Pro 5.4 (Molecular Devices, Inc.)

- 4) SigmaPlot (v. 11.0, Systat Software, Inc.)
- 5) Statistical Analysis Software (SAS v. 9.0, SAS, Inc.)

## **7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES**

Samples and standards are diluted and analyzed in the same plate.

Serial 2-fold dilution of standards: Standard vial is reconstituted in pfw according to manufacturer's instruction, vortex for 1 min and dilute in pfw.

A 12-step standard curve is generated with  $r^2 \geq 0.995$  based on maximum rate of change of OD with time for each standard. Control standard endotoxin is diluted in pyrogen-free 96-well microplates using pfw for the diluent. The concentrations of the 12 standards were 50.0, 25.0, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391, 0.195, 0.0977, 0.0488, 0.0244 EU/ml.

The sample dilutions for computing the sample endotoxin concentration are selected based on the standard curve and determined according to the following criteria. The values that fall within the optimal part of curve are evaluated for reporting. The optimal region of the sigmoidal standard curve is that region generally with a slope of the semi-log calibration plot greater than 20. Endotoxin values for samples at higher dilutions are generally selected for reporting to minimize assay interference. The data are entered to an Excel file formatted as specified by NHANES staff.

## **8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS**

### Preparation NHANES dust samples for analysis

A precise weight of NHANES dust of about 50 mg (xx.xx mg) is weighed in new 2mL pyrogen-free polypropylene vials with screw caps using a calibrated Mettler MT5 or Mettler XP26 microbalance in a temperature and humidity controlled gravimetric laboratory (room 194A IREH).

Dust aliquot vials are stored in plastic or fiberboard storage boxes.

Mettler-Toledo BalanceLink software is used to record dust weight and transfer data to an Excel file. All weights will also be recorded in the lab book labeled "NHANES". Weights are saved in an Excel file on the UI College of Public Health secure network at:

M:\General Research Directory\OEH-PTF\NHANES\DustWeights

This network is backed up daily and backups are securely archived.

**Endotoxin in dust  
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The microbalance is calibrated and certified at least annually. A copy of the calibration record is saved in the NHANES file folder.

Endotoxin and allergen extraction from the dust:

In a laminar flow hood, the appropriate volume of sterile pyrogen-free LAL water (pfw) plus 0.05% Tween 20 (pfw+T) is added to the NHANES dust so that the final concentration is 50.0 mg/ml (e.g. 50.00 mg dust + 1.00 ml pfw+T).

The dust is suspended by mixing for 1 min with a Vortex-Genie mixer (Fisher Scientific), shaken for 1 hr at room temperature on an orbital shaker (Fisher Scientific), centrifuge for 20 min at 600 xG and 4°C in a centrifuge ( Marathon 16KM, Fisher Scientific) to remove large insoluble particles from the eluate.

100.0 µl of the supernatant is transferred using a pipettor to a new cryovial for endotoxin analysis.

For dust samples weighing 50 mg, the 540.0 µl of supernatant using a pipettor is transferred to new cryovials and 60.0 µl of 10X pyrogen-free phosphate buffered saline is added and vortexed for 5 minutes. 200.0 µl is transferred to a new cryovial for shipment to Indoor Biotechnologies, Inc. (IBI) and the remaining extract (~400 µl) is transferred to a new cryovial for shipment to Air Quality Sciences, Inc. (AQS). For samples with less than 50 mg mass, aliquots are split equally for shipment to IBI and AQS.

The remainder of supernatant (~300 µl) and pellets are kept at -80°C as an archived sample.

All aliquots are stored at -80°C until they are shipped.

NHANES dust extracts for IBI are shipped frozen on dry ice in batches as follows:

Indoor Biotechnologies, Inc.  
1216 Harris Street  
Charlottesville, VA 22903  
Tel: (434) 984-2304

NHANES dust extracts for AQS are shipped frozen on dry ice in batches as follows:

Air Quality Sciences Inc.  
Sample Control - NHANES  
2211 Newmarket Parkway – Suite 106  
Marietta, GA 30067  
Tel: (770) 933-0638

Samples are shipped by FEDEX overnight on Mondays and Tuesdays only.

### **DUST SAMPLE WEIGHING**

This procedure is intended for use with the Mettler M5 Microbalance and the Mettler XP26 Microbalance as used for precision weighing of dust samples (readability 0.001 mg, precision ~0.002 mg). The microbalances are housed within a dedicated gravimetrics laboratory serviced by filtered and conditioned air. Temperature and relative humidity are maintained and monitored within established setpoints.

#### **Microbalance Cleaning:**

The microbalance pan and the weighing table around the microbalance is cleaned with a Kimwipe sprayed with 70% alcohol before and after each use. Avoid spraying alcohol on electronic equipment:

1. Remove the glass cover of the weighing chamber and clean thoroughly with a Kimwipe and 70% alcohol.
2. The weighing pan can be removed for cleaning or if desired, it can be lightly cleaned where it is located using a Kimwipe and 70% alcohol. If this is not done lightly, it will cause errors or disable the balance. To remove the pan for cleaning, use the tongs located in the small drawer on the left hand side of the display module. To remove the pan, lower the tongs into the weighing chamber vertically, gently grasp the edges of the pan with the tongs, lift straight up (DO NOT LIFT AT AN ANGLE) and slightly turn the tongs while lifting. Gently clean the pan and pan stem with a Kimwipe and 70% alcohol.
3. Remove the debris pan by carefully lifting it up through the top of the weighing chamber. Be careful not to allow any debris in the pan to fall off into the weighing chamber. Clean thoroughly with a Kimwipe and 70% alcohol.
4. Reverse the process by carefully replacing the debris pan, weighing pan, and glass cover. When replacing the weighing pan, simply insure that the pan stem is balanced in the center by using the tongs to place on the central holding nut (inside opening). Do not attempt to force the pan in place, do not twist the pan in place and do not attempt to thrust downward on the pan. When the balance is on, one can determine if the pan is placed correctly since the display will read "0.000". If the pan is not in place, the display will read "----".

During the course of weighing, it may be necessary to periodically clean the balance to remove excess debris. If you are weighing filters or other uncontained samples, the weighing pan must be washed in between samples. Wear gloves at all times. DO NOT BLOW INTO THE WEIGHING CHAMBER TO REMOVE DEBRIS.

#### **Microbalance Operation:**

1. The balance should be off when not in use. To turn the unit off, gently lift the tare bar. The display should read "Stand-By".
2. To turn on the balance, gently depress the display bar. The display will go through a sequence of self-checking and then the display should read "0.000". The menu settings are the standard settings for



the EHSRC PTF. To make adjustments using the display keys, consult the owner's manual. Return the balance to the default settings after use.

3. If the display does not read "0.000" with the weighing chamber closed, check to make sure there is nothing on the weighing pan and then gently press the display bar to tare the balance.
4. Every time the balance is used, the balance operation should be checked using the standard weights.
  - a. There are three standard weights; 200 mg, 100 mg, and 20 mg.
  - b. Do not handle the standard weights without using gloves and tweezers since the oils on your skin can influence the weight.
  - c. Record the weight of each standard in the spreadsheet on the computer hard-drive (see section on automatic recording via weighing software).
  - d. Return the standard weights to the small labeled vials after use.
  - e. On average, the difference between the scale reading and the standard weights should be  $\pm 0.005$  mg. If the average of the standard weights lies outside this range, clean the balance, tare, and reweigh the standards.
5. If the standard weights appear to be correct, the balance can now be used.
6. When weighing samples, gently place sample on the weighing pan. DO NOT DEPRESS PAN. Make sure the draft chamber is close and the sample weight has stabilized before recording weight.
7. Do not weigh any objects over 5 grams on the MT5 or 22 g on the XP26. Using the balance close to its maximum weight is not advised.
8. The weighing chamber draft shield is opened and closed using the two buttons on the display pad (one opens the shield to the right, the other to the left).

Autoweigh and Computer Recording:

1. The balance allows for automatic weighing. To do this, open the draft chamber, place the sample on the weighing pan, and depress the "print" key on the display pad.
2. This auto weigh can be automatically recorded using the software loaded on the computer next to the balance.
  - a. To turn on the software, log onto the computer using your University of Iowa, HawkID and password.
  - b. Open the BalanceLink software.
  - c. Open an Excel spreadsheet. This is where the weights are stored. When "print" is depressed, the software inputs the weight into the selected Excel cell once the sample weight reading is relatively stable (note: this take longer for some samples and sometimes it is faster to allow the sample weight to stabilize first before pushing the "print" button). It also inputs the unit of measure (mg) into the cell just to the right of the selected cell. Because of this, keep the column next to the recording column blank so as to not overwrite data.

3. Use the computer recording when weighing standard weights. An Excel spreadsheet has been developed for this purpose and can be found on the computer hard drive at:  
C:\Balance Calibration\Microbalance Calibration Data.xls  
Be careful not to write over previous data. Make sure you include the date, time, temperature, humidity, standard weight results, your full name, and the project acronym.
4. Always log off after using the microbalance.

Gravimetrics Laboratory Conditions:

1. The microbalance is used frequently. If you have a large number of samples to weigh, please sign up for a time and date on the calendar located on the door to the microbalance room.
2. No more than two people should occupy the Gravimetrics Lab while precision weighing is in process.
3. Samples are sensitive to temperature and humidity. To maintain the appropriate temperature and humidity conditions in the microbalance room the door must be kept closed.

Instrument Start up:

- Turn on reader and set temperature to 37°C
- Turn on computer and open Softmax Pro 5.4
- Click on protocol/ELISA-kinetic and set settings as follows:
  - Time: 1.33hr; Interval: 0.31min; Minimum Intervals: 31; Reads: 181
  - Automix: before: 5 sec and after: 3 sec; OD min: 0; OD max: 2.8; V max: 18
  - Wavelength: 405 nm
- Enter template as follows:
  - DS= NHANES dust sample; Std= Control Standard Endotoxin; High Cntl= High Endotoxin House Dust Control Sample; Low Cntl= Low Endotoxin House Dust Control sample; 1:400=1 to 400 dilution of samples.
- Microplates are loaded as follows:

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	1	2	3	4	5	6	7	8	9	10	11	12
A	High Cntl 1:400	Blank	Blank	DS 1 1:400	DS 3 1:400	DS 5 1:400	DS 7 1:400	DS 9 1:400	DS 11 1:400	DS 13 1:400	DS 15 1:400	DS 17 1:400
B	High Cntl 1:1600	Std 1	Std 7	DS 1 1:1600	DS 3 1:1600	DS 5 1:1600	DS 7 1:1600	DS 9 1:1600	DS 11 1:1600	DS 13 1:1600	DS 15 1:1600	DS 17 1:1600
C	High Cntl 1:6400	Std 2	Std 8	DS 1 1:6400	DS 3 1:6400	DS 5 1:6400	DS 7 1:6400	DS 9 1:6400	DS 11 1:6400	DS 13 1:6400	DS 15 1:6400	DS 17 1:6400
D	High Cntl 1:25600	Std 3	Std 9	DS 1 1:2560	DS 3 1:25600	DS 5 1:25600	DS 7 1:25600	DS 9 1:25600	DS 11 1:25600	DS 13 1:25600	DS 15 1:25600	DS 17 1:25600
E	Low Cntl 1:400	Std 4	Std 10	DS 2 1:400	DS 4 1:400	DS 6 1:400	DS 8 1:400	DS 10 1:400	DS 12 1:400	DS 14 1:400	DS 16 1:400	DS 18 1:400
F	Low Cntl 1:1600	Std 5	Std 11	DS 2 1:1600	DS 4 1:1600	DS 6 1:1600	DS 8 1:1600	DS 10 1:1600	DS 12 1:1600	DS 14 1:1600	DS 16 1:1600	DS 18 1:1600
G	Low Cntl 1:6400	Std 6	Std 12	DS 2 1:6400	DS 4 1:6400	DS 6 1:6400	DS 8 1:6400	DS 10 1:6400	DS 12 1:6400	DS 14 1:6400	DS 16 1:6400	DS 18 1:6400
H	Low Cntl 1:25600	Blank	Blank	DS 2 1:25600	DS 4 1:25600	DS 6 1:25600	DS 8 1:25600	DS 10 1:25600	DS 12 1:25600	DS 14 1:25600	DS 16 1:25600	DS 18 1:25600

- Samples and standards are diluted and analyzed in the same plate
- Serial 2-fold dilution of standards: Standard vial is reconstituted in pfw according to manufacturer's instruction, vortex for 1 min and dilute in pfw.
- A 12-step standard curve is generated with  $r^2 \geq 0.995$  based on maximum rate of change of OD with time for each standard. Control standard endotoxin is diluted in pyrogen-free 96-well microplates using pfw for the diluent. The concentrations of the 12 standards were 50.0, 25.0, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391, 0.195, 0.0977, 0.0488, 0.0244 EU/ml.
- Using a multichannel pipettor add 100 $\mu$ l of diluent (pfw) to each well of C2, D2, E2, F2, G2, B3, C3, D3, E3, F3, G3; then standard is reconstituted in recommended volume, vortex for at least 1 min.
- Using a single pipette, the following diluted standards are prepared:
  - Std1: 100 $\mu$ l of reconstituted standard is transferred to well B2
  - Std2: 100 $\mu$ l of reconstituted standard is transferred to well C2. Pipette up and down 5 times to rinse tip
  - Std3: With new tip, pipette up and down for 10 times and transfer 100 $\mu$ l from Std2 (well C2) to Std3 (well D2) and onward to Std12 then discard the final 100 $\mu$ l withdrawn from well G3
- Serial Dilutions of Samples: NHANES dust and bench control dust extracts are diluted 1:100, 1:400, 1:1000, 1:6400 and 1:25600 in pfw. 1:100 dilutions are performed in borosilicate glass tubes (depyrogenated by heating > 2 h in a 200°C oven) by combining 10.0 $\mu$ l of extract plus 990.0 $\mu$ l of LAL water. Remaining dilutions are performed in the microplate using single and multichannel channel pipettors.
- Using a multichannel pipettor 150.0 $\mu$ l of diluent is added to assigned sample wells
- Prepare 1:400 dilutions of samples: Vortex 1:100 dilution for 10 sec and use a single pipettor to transfer 50.0 $\mu$ l of 1:100 dilutions to 1:400 dilution wells. Pipette up and down 5 times to rinse tip
- Using multichannel pipette the following dilutions are prepared:
  - 1:1600 dilution: Pipette 1:400 wells up and down 5 times and discard 50 $\mu$ l. With new tip, pipette up and down 5 times and transfer 50.0 $\mu$ l of 1:400 dilutions to 1:1600 dilution wells. Pipette up and down 5 times to rinse tip and discard 50.0 $\mu$ l.
  - 1:6400 dilution: With new tip, pipette up and down 5 times and transfer 50.0 $\mu$ l of 1:1600 dilution to 1:6400 dilution wells. Pipette up and down 5 times to rinse tip and discard 50.0 $\mu$ l.
  - 1:25600 dilutions: with new tip, pipette up and down 5 times and transfer 50.0 $\mu$ l of 1:6400 dilution to 1:25600 dilution wells. Pipette up and down 5 times to rinse tip and discard 100.0 $\mu$ l.
- Plates are pre-incubated for 10 minutes at 37°C (incubator within the SpectraMax M5 Reader).

- Near the end of the preincubation period, LAL lysate is reconstituted in 2.6ml pfw and mixed gently.
- The entire lysate reagent is pooled into a reagent reservoir and mixed gently.
- 100.0 µl of reconstituted lysate is dispensed to each well using a multichannel pipettor.
- The microplate is inserted into the SpectraMax M5 Reader and read as described above.

Calculations:

Continuously throughout the assay, the microplate reader is monitoring the absorbance at 405 nm of each well of the microplate, using the initial absorbance reading of each well as its own blank. SoftMax Pro software automatically performs a log/log linear correlation of the reaction time of each standard with its corresponding endotoxin concentration. Four-parameter logistic log fit of mean rate of change of optical density, Y (milliOD/min) as a function of endotoxin concentration, X (EU/mL) follows the equation below. A and D are the lower and upper asymptotes. B is the slope factor and C the inflection point.

$$Y = \left( \frac{A - D}{1 + \left(\frac{X}{C}\right)^B} \right) + D$$

Blank wells test the endotoxin-free status of the pfw. The sample dilutions for computing the sample endotoxin concentration are selected based on the standard curve and determined according to the following criteria. The values that fall within the optimal part of curve are evaluated for reporting. The optimal region of the sigmoidal standard curve is that region generally with a slope of the semi-log calibration plot greater than 20. Endotoxin values for samples at higher dilutions are generally selected for reporting to minimize assay interference. The data are entered to an Excel file formatted as specified by NHANES staff.

## **9. REPORTABLE RANGE OF RESULTS**

The clinical significance of endotoxin concentrations in house dust has not been established. The reportable range is within the range of the standard curve.

## **10. QUALITY CONTROL (QC) PROCEDURES**

The following QA/QC samples are analyzed:

- Random repeat analysis of ~5% of samples on different microplates and different days;
- One High Endotoxin House Dust Bench Control (High Control) sample and one Low Endotoxin House Dust Bench Control (Low Control) sample for each day; and
- Repeat runs of NHANES dust samples that fail QC rules or that fall beyond the limits of the standard curve.

High and low bench control samples were prepared by the PTF staff and remain the property of the University of Iowa for use in this and other studies. They were created by blending 50 selected reservoir household dust samples collected from Iowa homes in two prior studies.

- High Endotoxin House Dust Bench Control samples: Sieved dust samples collected from the Louisa/Keokuk Environmental Intervention Project (LEIP) on Rural Childhood Asthma were pooled to create the high endotoxin bench control samples. Using special vacuums and HEPA socks, dust samples were collected from child bedroom bedding, child bedroom floors, child play area floors, and kitchen floors in Southern Iowa homes of asthmatic children. Dust samples were collected before and on three occasions after home hygiene educational and extensive professional cleaning interventions. Samples were sieved using 45 mesh size and stored at -20°C. A total mass of 23.0 g was prepared.
- Low Endotoxin House Dust Control samples: Samples collected from the Louisa/Keokuk Environmental Intervention Project (LEIP) on Rural Childhood Asthma and the Rural Childhood Asthma Study (RCAS) were pooled as described above to generate the low endotoxin bench control samples. Samples were sieved using 45 mesh size and stored at -20°C. A total mass of 32.0 g was prepared.

Preparation High and Low Control Dusts:

- Dust was blended and re-sieved using 45 mesh size.
- 50.0 mg aliquots of dust were weighed into 1,100 cryovials, labeled and stored frozen in cryovial boxes.
- One vial is used for each microplate in the NHANES analyses.
- Extraction is performed as with NHANES dust samples.

Quality Assurance:

QA/QC measures included the use of blanks, high and low endotoxin bench control samples and a 12-point standard curve on each microplate. In addition, blind random repeat samples provided by NHANES are analyzed. An external QA audit is performed by NHANES. Dr. Andrea Dodd serves as the internal QA auditor. She observes all lab staff performing their work and ensures compliance with SOPs. In addition, she reviews lab books, analysis details and data entry.

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

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.

## **12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS**

The work area is cleaned between processing of each dust sample to preclude cross-contamination.

Dust samples are weighed and sieved under a laminar flow hood.

## **13. REFERENCE RANGES (NORMAL VALUES)**

There are no established ranges for this type of testing.

## **14. CRITICAL CALL RESULTS ("PANIC VALUES)**

There are no critical calls for this type of testing.

## **15. SPECIMEN STORAGE AND HANDLING DURING TESTING**

Dust is stored in dry, room temperature conditions or cooler. Eluates are stored at -20°C.

## **16. 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 eluate at -20°C until the system is functioning.

## **17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)**

Not applicable.

## **18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING**

Maintain residual samples from these assays in the same freezer that is used to hold samples waiting to be processed. Periodically, transfer racks of completed samples to dense-pack boxes that are labeled for the contents and transferred for archive storage in a -70° C freezer. Samples are stored by Study name and run ID number. This information along with the freezer ID number and location is maintained in an Excel file for subsequent tracking purposes.

**19. SUMMARY STATISTICS AND QC GRAPHS**

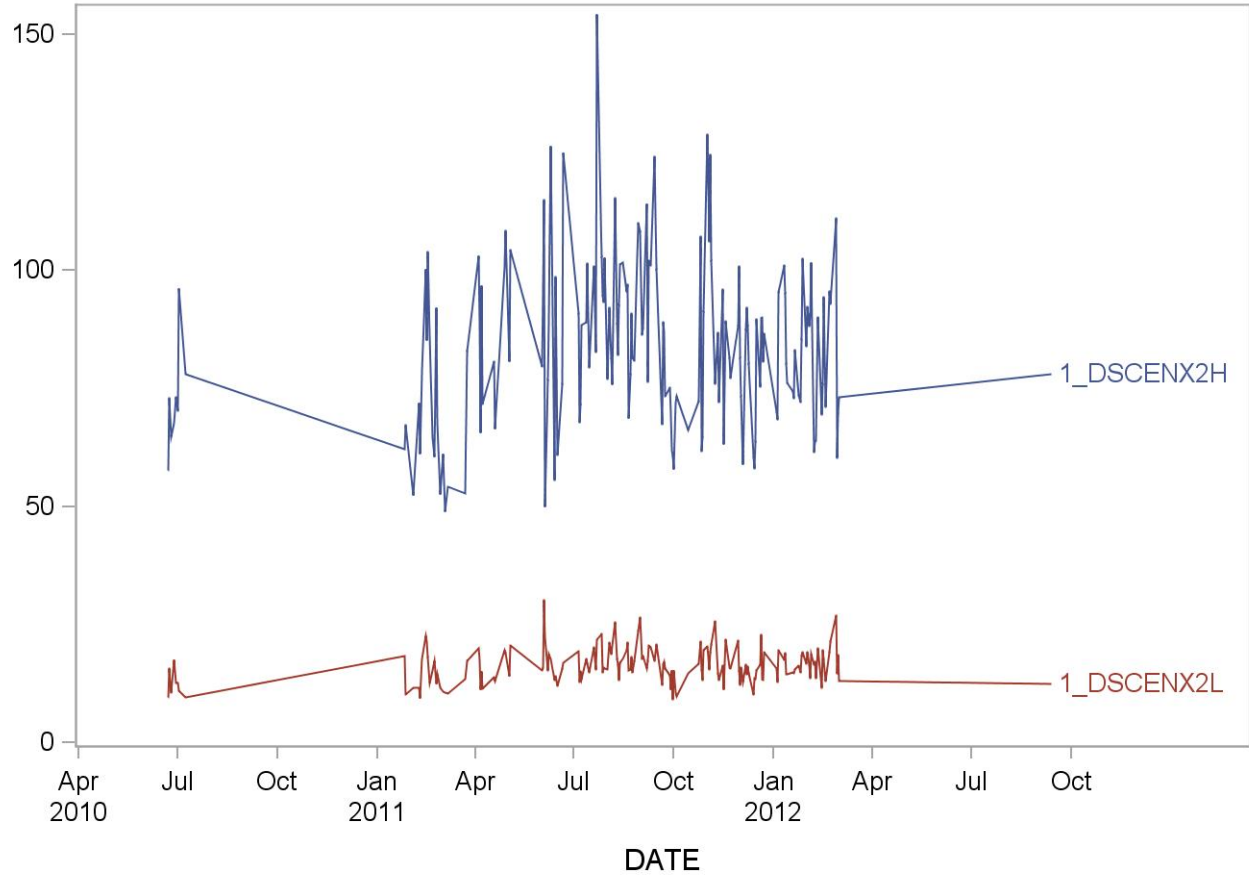
See following pages.



### Summary Statistics for Dust Endotoxin (EU/mL)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
1_DSCENX2H	373	22JUN10	12SEP12	84.0682	19.9953	23.8
1_DSCENX2L	376	22JUN10	12SEP12	16.5296	4.5244	27.4

### 2005-2006 Dust Endotoxin (EU/mL) Quality Control



**REFERENCES**

None