Laboratory Procedure Manual

Analyte: Aspergillus fumigatus

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.
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Public Release Data Set Information

This document details the Lab Protocol for NHANES 2005–2006 data.

A tabular list of the released analytes follows:

<table>
<thead>
<tr>
<th>File Name</th>
<th>Analyte</th>
<th>SAS Label</th>
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<tbody>
<tr>
<td>ALDUST_D</td>
<td>DSXAF1</td>
<td>Aspergillus fumigatus Results (ng/mL)</td>
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</table>
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**PROTOCOL FOR ASPERGILLUS**

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

   This method describes the procedure for measuring amounts of *Aspergillus fumigatus* antigens in samples, using custom-prepared, antiserum enzyme-linked immunosorbent assay (ELISA) test from Greer Laboratories (Lenoir, NC). This ELISA test provides a quantitative measure for all antigens that bind to the custom antiserum. 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 antigen. The concentration is determined by measuring color intensity at a specific wavelength.

   The test is to be conducted by qualified analysts who have received training on dust mold analysis by ELISA assay.

   *Aspergillus fumigatus* is a common outdoor mold associated with decaying vegetative matter. It is one of the molds commonly associated with allergic and/or asthmatic symptoms. From a clinical perspective, the data can be used to either validate or establish the mold 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 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 each sample within a set of samples. Individual samples (specimens) 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. *Aspergillus fumigatus* antigen concentrations are computer generated by the proprietary software provided by the instrument manufacturer (Molecular Devices). The software compares test values with the control curve. Samples with test values below the low cutoff value are reported as below limit of detection, while samples with test values for the highest dilution above the high cutoff value are retested with one increased set of dilutions. 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 CDC upon the completion of allergen and mold antigen tests for each sample.

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

   A. Samples 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 or wet are rejected and the CDC will be notified in the final report.

   C. Samples are stored at -20°C until used.

   D. If less than 60 µl of eluate sample (20µl for Bla g 1 and 25ul for *Aspergillus fumigatus* is obtained, 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*).
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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.

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

A. The following instrumentation is employed to analyze dust molds at AQS:
1. SpectraMax 340pc (Molecular Devices, Sunnyvale, CA)
2. SoftMax 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 micropipetter (Fisher Scientific, Suwanee, GA, Cat. No. 05-402-88)
2. Eppendorf Research Pro 100 µL single channel micropipetter (Fisher Scientific, Suwanee, GA, Cat. No. 22-46 131-1)
3. Eppendorf 1000 µL single channel micropipetter (Fisher Scientific, Suwanee, GA, Cat. No. 05-402-90)
4. Eppendorf Research Pro 300 µL multi-channel micropipetter (Fisher Scientific, Suwanee, GA, Cat. No. 22-46 142-7)
6. 10 mL serological pipettes (Fisher Scientific, Suwanee, GA, Cat. No. 13-676-10J).
7. 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).
13. Plate Washer machine (Molecular Devices).

C. Reagent preparation

1. All antibodies and standards for Aspergillus fumigatus antigen are manufactured by Greer Laboratories, Inc.
2. Antigens and standards are prepared according to manufacturer’s instructions.
3. Recipes for all ELISA solutions and reagents are located in the Microbiology Laboratory's Media book.
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.)

50mM CARBONATE-BICARBONATE BUFFER, pH 9.6 +/- 0.2
Na₂Co₃ 1.59 g
NaHCO₃ 2.93 g
Thimerosal (Optional) 0.1mL
Distilled Water 1000 mL

PHOSPHATE BUFFERED SALINE WITH 0.05% TWEEN 20, pH 7.4 +/- 0.2 (PBS-T)
NaCl 8 g
KH₂PO₄ Monobasic 0.2 g
Na₂HPO₄ 1.15 g
KCl 0.2 g
Thimerosal (Optional) 0.1mL
Tween 20 (50%) 1mL
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Distilled Water 1000 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

DIETHANOLAMINE (DEA) BUFFER pH 9.8 +/-0.2
MgCl₂ 0.101 g
Diethanolamine 98% 97 ml
HCl 12.1N 7.5 ml
Distilled water 1000 ml

P-NITROPHENYL PHOSPHATE SUBSTRATE: Amresco Item # 0364-25G.

AVIDIN-ALKALINE PHOSPHATASE CONJUGATE: Zymed, Item # 43-4422.

D. Preparation of quality control material

1. Two “bench control” samples are included in each microplate. The bench controls are targeted for the midrange-to-bottom and midrange-to-top of the linear portion of the standard curve.
2. The positions of the bench control samples on the microplate are generated randomly.
3. 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 are prepared in duplicate on the plate.
3. Concentrations range as follows: 1220–2.383 ng/mL.
4. Absorbance values of the calibration wells are regressed on the concentration.
5. A sigmoidal 4-point curve fit was used for this assay. Calibration curves are inspected to establish the linear portion of the curve and to verify that the curve is based on a minimum of five (5) points, the regression has a r-squared value of no less than 0.995, and the working lower limits of detection (LLOD) of less than or equal to 35 ng/mL of Aspergillus fumigatus antigen. The LLOD is the calculated concentration of the lowest point on the linear portion of the standard curve multiplied by the lowest dilution factor (8).

7. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

Procedure

A. Coating of Microplates
1. Label each Nunc 96 well microplates with date and test type.
2. Prepare coating solution by adding 10μL of Capture Antibody (α-Aspergillus fumigatus, Greer) to 10ml carbonate-bicarbonate solution at 1:1000 ratio.
3. Dispense 100 μL of coating solution to all wells.
4. Incubate overnight at 25°C.
5. Record the reagent lot numbers on the ELISA worksheet.

B. Wash
1. Remove coated microplate(s) from 25°C incubator.
2. Wash all wells 3x with 300μl of PBS-T.

C. Addition of Blocking Agent
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1. Dispense 200 µL of 1%BSA-PBS-T (see 5.C) to each well.
2. Incubate for 30 min. at room temperature (20-25 °C).
3. Record the reagent lot numbers on the ELISA worksheet.

D. Wash

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

E. Loading and Dilution of Standard

1. Standard curve use twenty wells on the microplate. Each standard is run in duplicate and diluted by ten serial two-fold dilutions.
2. Prepare standard solution 1:25 dilution by adding 40µl of Asp. fumigatus #6 extract to 960µl 1%BSA-PBS-T with 50% Glycerol(1:25 i.e. 12200ng/ml).
3. Add 100 µL of PBS-T to all standard curve wells.
4. Add 80µL of PBS-T to wells A1 and B1.
5. Add 20µL of standard solution to wells A1 and B1.
6. Transfer 100 µL from wells A1 and B1 to wells A2 and B2, respectively.
7. Continue transfer and suspension of 100 µL through wells A10 and B10. Mix thoroughly prior to each transfer.
8. Discard 100 µL from wells A10 and B10.
9. Record the reagent lot numbers on the ELISA worksheet.

F. Sample and bench controls Loading and Dilution

1. Dispense 100µL of PBS-T to all sample wells.
2. Add 75 µL of PBS-T wells (C1-H1, C4-H4, C7-H7 and C10-H10).
3. Add 25 µL of eluate from each sample individually to a sample well (C1-H1, C4-H4, C7-H7 and C10-H10). Mix thoroughly before transfer.
4. Transfer 100µL of 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 100µL from the second dilution well. Repeat for the other sample wells. Initial dilutions are 1:8, 1:16 and 1:32.
5. Pipette out all the PBS-T from bench control assigned wells. Load bench control samples by dispensing 100 µL of appropriate bench control solution to well(s) assigned randomly. Bench control samples are not diluted.
6. Incubate plates for 1.5 hours at 20-25 °C.

G. Wash

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

H. Preparing and loading Biotinylated probe Antibody (2nd AB)

1. Prepare secondary AB solution @1:1000 (10µl/10ml) in 1%BSA-PBS-T.
2. Load 100µl to all wells.
3. Incubate the plate for 2.5 hours at 20-25 °C.

I. Preparing and Loading Conjugate

1. Prepare conjugate solution at a 1:2000 concentration by diluting avidin-alkaline phosphatase conjugate (5µl/10ml) with 1%BSA-PBS-T.
2. Dispense 100 µL of solution to all wells.
3. Incubate microplate(s) for one hour at 20-25 °C.
4. Record the reagent lot numbers on the ELISA worksheet.

J. Wash
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1. Wash all wells 3x with 300 µl of PBS-T.
2. Add 150 µl of DEA buffer and blot dry after 2-3 minutes.

K. Addition of Substrate

1. Prepare substrate solution by adding p-NPP powder to DEA buffer at (10 mg per 10 mL).
2. Dispense 100 µL of substrate solution to all wells.
3. Observe color change from clear to yellow.
4. Read plate on SpectraMAX 340pc plate reader at 405nm.
5. Approximate development time is 30-40 minutes.
6. 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 1.0 and 1.2.

L. 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 at 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 mold folder of the current year.
6. Open the mold 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 and 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 1.0 and 1.2, save the data in the file and name it as S/YY/MM/DD/Plate number.
12. Save the file in its appropriate folder.
13. New ELISA and mold folders are to be created at the beginning of the year, or more frequently as appropriate.

M. 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 25.0 or under.
4. Standard curves must contain at least five, in contiguous, dilution points.
5. The Back Calc Concentration of the highest dilution should be low enough for the LLOD to be less than or equal to 11.3 ng/ml of Aspergillus fumigatus antigen.
6. If the working LLOD is higher than this value, then the plate must be rejected and repeated.
7. Print out the edited datasheet in portrait form and attach to ELISA worksheet.

N. Analyzing the Datasheet From SoftMax Pro

1. Standards and Plate Settings
   a. 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.
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b. 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.

c. Check the CV% values of the standards and underline any that are over 25.

d. Note and circle 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.

e. Note and circle the standard curve R^2 value.

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") and not reported.

b. The result(s) not rejected are evaluated for reporting. The result, average value from all dilutions in range is reported.

c. If two or more R’s are present, AQS software then 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 Aspergillus fumigatus antigen 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 ULOD (upper limit of detection) is reported and annotated with code “102”.

d. Staple the ELISA Worksheet created for the mold 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 then record the reasoning on the ELISA Worksheet.

f. If the plate is to be used with R^2 out of the normally accepted range, then state why on the ELISA Worksheet.

O. Transferring Data from SoftMax Pro to Report Spreadsheet

Upon completion of each Aspergillus fumigatus antigen test plate read, the data is saved in ADM. A barcode system used to assign each dust 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 ADM for the presence of a new mold test plate file, and when found, it will extract the information into the e-file. The AQS barcode number is also 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 reject. If more than one result is accepted, then the average value of these results will be reported. If no samples are accepted, the routine determines whether the cause is due to all values being less than the LLOD or no test performed due to insufficient sample volume. If the reason is all values less than the LLOD, the LLOD value and comment code 37 is recorded. If the reason is insufficient sample volume, comment code 21 is recorded. Once all items on an e-file have been completed, an internal notification e-mail advises AQS personnel that a new e-file is ready for review.

P. Calculations

1. Calculations are computerized within SoftMax Pro.

2. Aspergillus antigen is reported as ng/ml (nanograms of mold per milliliter of sample).

3. To obtain the mold concentration, calculations are completed through SoftMax Pro as follows:
   a) Multiply the adjusted results with the dilution factor.
   b) Take the average of all in range results of each sample.
   c) Round the result to two decimal points.
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**Quality Control Materials**

Two bench control samples are included in each run in the same manner as a sample. 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 (1:8, 1:16, and 1:32) is equal to or above LLOD value of *Aspergillus* antigen in ng/ml of sample eluate. Higher concentrations are determined through additional dilutions of the sample and adjustment of the accepted result by the dilution factor. Only the linear portion of the standard curve is used, therefore, reported values are from sample dilutions that fall within the linear portion of the standard curve. Due to the intrinsic variability from plate to plate, a lower limit of detection is determined individually for each plate. Typically, values less than LLOD are reported and flagged with comment code “37” and the LLOD for the plate is reported. Values greater than 80% of the highest value on the standard curve are assayed with further dilution (1:64, 1:128, 1:256). Samples with values greater than the upper limit of detection for the 2nd assay are reported with comment code “102” along with the upper limit of detection for the higher dilution 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 QC control charts are used for monitoring the following QC results for each plate run: bench control concentrations (ng/mL) and the Lower Limit of Detection (LLOD) for the bottom of the standard curve. Operating Characteristic Charts are maintained for the slope and intercept of the standard curve and standard curve linearity. 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 ± 2s of the fixed mean value determined from inter-plate and intra-plate 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
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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 between processing of each dust sample to preclude cross-contamination.
B. Microplates are covered during incubation periods to prevent dust or particulate matter from settling into wells and compromising assay results.
C. Fingerprints or other markings from lab bench and area may interfere with reading of microplates. The bottom and sides of each plate in wiped down with a Kimwipe and 70% Isopropanol immediately before plate is placed into spectrophotometer.
D. Concentration of mold in a sample in the highest dilution normally created on a plate may be higher than the largest standard value. The sample must then be set up on a new ELISA plate, diluted out further, and the calculations adjusted to meet new dilution factor.
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. 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. There is no established Aspergillus fumigatus antigen level associated with an increased risk of sensitization among atopic individuals.

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

Sample Eluates are stored at -20°C.

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 eluate 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 Aspergillus Fumigatus (ng/mL)

<table>
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DATE

- Mar 2011
- May
- Jul
- Sep
- Nov
- Jan 2012
- Mar
- May
- Jul
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REFERENCES

- Test kit insert, Greer Laboratories, Lenoir, NC, 2010.