



Laboratory Procedure Manual

Analyte: **Specific IgE / Total IgE**

Matrix: **Serum**

Method: **Pharmacia Diagnostics
ImmunoCAP 1000**

Method No:

Revised: 3/24/2006,9/12/2006

as performed by:

Immunology Laboratory
Department of Pathology
Elmhurst Memorial Hospital

contact:

Dr. Ronald M Lam, Chairman
Department of Pathology and
Laboratory Medicine
Elmhurst Memorial Hospital

June 2008

Important Information for Users

Elmhurst Memorial Hospital 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.

**Specific IgE/Total IgE Allergens in Serum
NHANES 2005-2006**

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

A tabular list of the released analytes follows:

File Name	Variable Name	SAS Label
AL_IGE_d	LBXIW1	Ragweed IgE antibody (kU/L)
	LBXIG5	Rye grass IgE antibody (kU/L)
	LBXIG2	Bermuda grass IgE antibody (kU/L)
	LBXIT7	Oak IgE antibody (kU/L)
	LBXIT3	Birch IgE antibody (kU/L)
	LBXF24	Shellfish IgE antibody (kU/L)
	LBXIM3	Aspergillus IgE antibody (kU/L)
	LBXW11	Thistle IgE antibody (kU/L)
	LBXE72	Mouse IgE antibody (kU/L)
	LBXE74	Rat IgE antibody (kU/L)
	LBXIGE	Serum total IgE antibody (kU/L)
	LBXID2	D. Farinae IgE antibody (kU/L)
	LBXID1	D. Pteronyssinus IgE antibody (kU/L)
	LBXIE1	Cat IgE antibody (kU/L)
	LBXIE5	Dog IgE antibody (kU/L)
	LBXII6	Dog IgE antibody (kU/L)
	LBXIM6	Cockroach IgE antibody (kU/L)
	LBXF13	Alternaria IgE antibody (kU/L)
	LBXF13	Peanut IgE antibody (kU/L)
	LBXIF1	Egg IgE antibody (kU/L)
LBXIF2	Milk IgE antibody (kU/L)	

Specific IgE/Total IgE Allergens in Serum

NHANES 2005-2006

1. Summary of Test Principle and Clinical Relevance

Anaphylaxis (Type 1 Hypersensitivity) is the clinical response to immunologic formation and fixation between a specific antigen and a tissue-fixing antibody. This is also known as immediate hypersensitivity because the reaction occurs within minutes of contact with the antigen or allergen. This reaction is usually mediated by IgE antibody and occurs in three stages: 1. The offending antigen attaches to the IgE antibody fixed to the surface membrane of mast cells and basophils. 2. Activated mast cells and basophils release various mediators, which are stored in their granules including histamine and heparin. 3. The effects of mediator release produce vascular changes, activation of platelets, eosinophils and neutrophils, synthesis of new mediators (leukotrienes) and activation of the coagulation cascade.

Anaphylactic reactions are dramatic and rapid in their onset. Histamine causes contraction of the bronchioles and smooth muscle of blood vessels, increases capillary permeability, and increases mucous gland secretion in the airway. Symptoms of an allergic reaction can include respiratory symptoms such as rhinitis and asthma, urticaria, angioedema, swelling of the bowel or systemic anaphylaxis. Hypersensitivities caused by IgE, regardless of their severity, depend upon the presence of an IgE with a serologic specificity for the offending allergen. Some non-allergenic environmental factors that elicit symptom in-patients with asthma and allergic rhinitis might erroneously be regarded as allergens.

This assay is intended for *in vitro* diagnostic use as an aid in the clinical diagnosis of IgE mediated allergic disorders in conjunction with other clinical findings.

A. Test principle

1. Specific IgE

The specific allergen of interest, covalently coupled to ImmunoCap cellulose carrier (sponge), reacts with the specific IgE in the patient serum sample. After washing away non-specific IgE, enzyme-labeled antibodies against IgE are added to form a complex. After incubation, unbound enzyme anti-IgE is washed away and the bound complex is then incubated with a developing agent. After stopping the reaction, the sponge is compressed and the fluorescence of the resulting eluate is measured. The higher the response value, the more specific IgE is present in the sample. To evaluate the test results, the response for the patient samples is compared directly to the response for the calibrators.

2. Total IgE

Anti-IgE, covalently coupled to ImmunoCap reaction vessel, reacts with the total IgE in the sample. After washing, enzyme-labeled antibodies against IgE are added to form a complex. After incubation, unbound enzyme-anti-IgE is washed away and the bound complex is then incubated with a developing agent. After stopping the reaction, the fluorescence of the eluate is measured. The fluorescence is directly proportional with the concentration of IgE in the sample. To evaluate the test results, the response for the patient samples is compared directly to the response for the calibrators.

2. Safety Precautions

Treat all serum specimens as potentially positive for infectious agents including HIV and hepatitis B. Observe Universal Precautions; wear protective gloves and lab coat during all steps of this method because of infectious contamination hazards. Recommend the hepatitis B vaccine series for all analysts working with intact blood and serum sample materials. Place all plastic and glassware that contacts serum in a Biohazard labeled plastic autoclave bag for disposal. Material safety data sheets (MSDS) for all chemicals contained in the kit are available in the MSDS book located in the laboratory. MSDS for other chemicals can be obtained by calling 1-800-451-8346 with the product name and manufacturer name.

Quality control materials contain reagents manufactured from human blood components. The source materials have been tested by immunoassay for hepatitis B surface antigen, for antibodies to HIV1, HIV2 and hepatitis C virus and found negative. Nevertheless, all recommended OSHA precautions for the handling of blood derivatives should be observed.

Reagents that contain sodium azide as preservative must be handled with care. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

3. Computerization; Data System Management

- A. ImmunoCap 1000 is interfaced with the Laboratory Information System (LIS). Weekly test result information is extracted from the LIS and placed into an Access database using ODBC drivers. Using the Access database an Excel spreadsheet is created containing all the required information on any completed sample person. This spreadsheet is sent by e-mail to Westat's ISIS computer system.
- B. Files stored on the LIS system are mirrored to an auxiliary server, and automatically backed up nightly by LAN support staff.
- C. Documents for data system maintenance are contained in the Information System department.

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

- A. No specific patient preparation is required for sample collection.
- B. 40 μ L serum for each allergen and 100 μ L dead space volume is required for each vial.
- C. Minimum amount of serum needed for each CDC Adult Panel:
19 Specific Allergens and 1 Total IgE = 20 μ L * 40 μ L = 800 μ L + 100 μ L dead space volume = 900 μ L
Minimum amount of serum needed for each CDC Pediatric Panel:
9 Specific Allergens + 1 Total IgE = 10 μ L * 40 μ L = 400 μ L + 100 μ L Dead Space Volume = 500 μ L
To perform 2% repeats after initial testing for Adult panel requires 900 μ L + 800 μ L = 1700 μ L
To perform 2% repeats after initial testing for Pediatric panel requires 500 μ L + 400 μ L = 900 μ L Serum.
- D. Serum specimens from venous or capillary blood collected in regular red top or Serum separator vacutainers can be used. Keeping the blood at room temperature for 30-45 minutes prior to centrifugation will help avoiding the fibrin clot in the serum sample. The appropriate amount of serum is dispensed into a Nalge cryovial or other plastic screw-capped vial labeled with the participant's ID.
- E. Specimens collected in the field should be frozen and then shipped on dry ice by overnight mail. Once received, they should be stored at -70 C until analyzed. Serum allergen values are fairly stable if the serum is frozen at -70 C before analysis.
- F. Specimens should generally arrive frozen. Refrigerated samples may be used provided they are brought promptly from the site where the blood was collected. Samples are stable at 2-8 C up to one week. Avoid repeated freezing and thawing.
- G. Specimens are rejected if received unfrozen, leaking or if the ID on the vial does not match the ID on the manifest.

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
 - 1. UniCAP/Pharmacia CAP System™ Washing Solution
Product No. 10-9202-01, 2 bottles (400 ml each) of Concentrate, 2 bottles (86 ml each) of additive.
Additive contains Surfactant and 5.8 % Kathon CG.
Concentrate contains Phosphate buffer and 0.05 % Kathon CG.
Store concentrate and additive at 2-8°C until the expiration date.
Reconstitute using 5 liters of distilled water, 1 bottle Concentrate and 1 bottle Additive. Mix thoroughly.
Prepared wash solution is stable for one week at room temperature.
- B. Standards Preparation

Not applicable for this procedure.

Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

C. Preparation of Quality Control Materials

Quality control materials purchased from Pharmacia Diagnostics are ready to use. No preparation required.

D. Other Materials

1. UniCAP Specific IgE Conjugate 400
Product No. 10-9310-02, 6 vials (20.5 ml each) x 400 determinations per vial.
B-Galactosidase-anti-IgE (mouse monoclonal antibodies) approximately 1microgram/ml
Sodium Azide 0.06 %. Ready to use. Store at 2-8°C until the expiration date. Do not freeze. On board open vial expiration date is 5 days. Pooling reagents is not recommended.
2. Unicap Total IgE Conjugate 100
Product No. 10-9319-01, 6 vials (5.3 ml each) x 100 determinations per vial. B-Galactosidase-anti-IgE (mouse monoclonal antibodies), Sodium Azide 0.06%. Ready to use. Store at 2-8 °C until the expiration date. On board open vial expiration date is 5 days. Pooling reagents is not recommended.
3. Development Solution
Product No. 10-9439-01, 6 bottles (65 ml each) x 1200 determinations per bottle
4-Methyumbelliferyl-B-D-Galactosidase 0.01 %, Kathon CG 0.05 %
Ready to use. Store at 2-8°C until the expiration date. Do not freeze. On board open bottle expiration date is 14 days. Pooling reagents is not recommended
4. UniCAP Stop Solution
Product No. 34-2271-51, 1 bottle (850 ml) x 1200 determinations per bottle.
Sodium Carbonate 4 %, Ready to use. Store at 2-8°C until the expiration date. Do not freeze.
On board open bottle expiration date is 14 days. Pooling reagents is not recommended.
5. UniCAP IgE/ECP/Tryptase Sample Diluent
Product No. 10-9256-01, 6 bottles of 3 ml, Chicken serum and bovine serum albumin.

Ready to use. Store at 2-8°C until the expiration date. Do not freeze.
6. Allergen-Specific ImmunoCAP (Specific IgE)
 - a. d1 – Dermatophagoides pteronyssinus – House dust mite
Product No. 14-4107-01. 16 ImmunoCAP per Carrier. Single allergen ImmunoCAP covalently coupled to cellulose solid phase, Kathon CG 0.15 %. Store at 2-8°C until the expiration date.
 - b. d2 – Dermatophagoides farinae – House dust mite
Product No. 14-4108-01. 16 ImmunoCAP per Carrier. Single allergen ImmunoCAP covalently coupled to cellulose solid phase, Kathon CG 0.15 %. Store at 2-8°C until the expiration date.
 - c. e1 – Cat Dander
Product No. 14-4109-01. 16 ImmunoCAP per Carrier. Single allergen ImmunoCAP covalently coupled to cellulose solid phase, Kathon CG 0.15 %. Store at 2-8°C until the expiration date.
 - d. e5 –Dog Dander
Product No. 14-4110-01. 16 ImmunoCAP per Carrier. Single allergen ImmunoCAP covalently coupled to cellulose solid phase, Kathon CG 0.15 %. Store at 2-8°C until the expiration date.
 - e. e72 – Mouse urine proteins
Product No. 14-4395-01. 10 ImmunoCAP per Carrier. Single allergen ImmunoCAP covalently coupled to cellulose solid phase, Kathon CG 0.15 %. Store at 2-8°C until the expiration date.
 - f. e74 – Rat urine proteins
Product No. 14-4398-01. 10 ImmunoCAP per Carrier. Single allergen ImmunoCAP covalently coupled to cellulose solid phase, Kathon CG 0.15 %. Store at 2-8°C until the expiration date.
 - g. f1 - Egg white

Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

- Product No. 14-4111-01. 16 ImmunoCAP per Carrier. Single allergen ImmunoCAP covalently coupled to cellulose solid phase, Kathon CG 0.15 %. Store at 2-8°C until the expiration date.
- h. f2 - Milk
Product No. 14-4112-01. 16 ImmunoCAP per Carrier. Single allergen ImmunoCAP covalently coupled to cellulose solid phase, Kathon CG 0.15 %. Store at 2-8°C until the expiration date.
- i. f13 – Peanut – *Arachis hypogaea*
Product No. 14-4126-01. 16 ImmunoCAP per Carrier. Single allergen ImmunoCAP covalently coupled to cellulose solid phase, Kathon CG 0.15 %. Store at 2-8°C until the expiration date.
- j. f24 – Shrimp – *Pandalus borealis*
Product No. 14-4181-01. 16 ImmunoCAP per Carrier. Single allergen ImmunoCAP covalently coupled to cellulose solid phase, Kathon CG 0.15 %. Store at 2-8°C until the expiration date.
- k. g2 – Bermuda Grass – *Cynodon dactylon*
Product No. 14-4131-01. 16 ImmunoCAP per Carrier. Single allergen ImmunoCAP covalently coupled to cellulose solid phase, Kathon CG 0.15 %. Store at 2-8°C until the expiration date.
- l. g5 – Rye-Grass *Lolium perenne*
Product No. 14-4134-01. 16 ImmunoCAP per Carrier. Single allergen ImmunoCAP covalently coupled to cellulose solid phase, Kathon CG 0.15 %. Store at 2-8°C until the expiration date.
- m. i6 – Cockroach – *Blattella germanica*
Product No. 14-4224-01. 16 ImmunoCAP per Carrier. Single allergen ImmunoCAP covalently coupled to cellulose solid phase, Kathon CG 0.15 %. Store at 2-8°C until the expiration date.
- n. m3 – *Aspergillus fumigatus*
Product No. 14-4119-01. 16 ImmunoCAP per Carrier. Single allergen ImmunoCAP covalently coupled to cellulose solid phase, Kathon CG 0.15 %. Store at 2-8°C until the expiration date.
- o. m6 – *Alternaria alternata* – *A tenuis*
Product No. 14-4106-01. 16 ImmunoCAP per Carrier. Single allergen ImmunoCAP covalently coupled to cellulose solid phase, Kathon CG 0.15 %. Store at 2-8°C until the expiration date.
- p. t3 – Common Silver Birch – *Betula verrucosa*
Product No. 14-4102-01. 16 ImmunoCAP per Carrier. Single allergen ImmunoCAP covalently coupled to cellulose solid phase, Kathon CG 0.15 %. Store at 2-8°C until the expiration date.
- q. t7 – Oak – *Quercus alba*
Product No. 14-4149-01. 16 ImmunoCAP per Carrier. Single allergen ImmunoCAP covalently coupled to cellulose solid phase, Kathon CG 0.15 %. Store at 2-8°C until the expiration date.
- r. w1 Common Ragweed – *Ambrosia elatior*
Product No. 14-4162-01. 16 ImmunoCAP per Carrier. Single allergen ImmunoCAP covalently coupled to cellulose solid phase, Kathon CG 0.15 %. Store at 2-8°C until the expiration date.
- s. 19. w11 - Russian Thistle – *Salsola kali*
Product No. 14-4249-01. 16 ImmunoCAP per Carrier. Single allergen ImmunoCAP covalently coupled to cellulose solid phase, Kathon CG 0.15 %. Store at 2-8°C until the expiration date.
7. Specific IgE - Anti-IgE ImmunoCAP
Product No. 14-4417-01, 16 ImmunoCAP per Carrier
S: a-IgE (mouse monoclonal antibodies) Mouse monoclonal antibodies with 0.15% Kathon®CG covalently bound to a cellulose solid phase within a holder. Store at 2-8°C until the expiration.
8. Total IgE anti – IgE ImmunoCAP
Product No. 14-4509-01
a-IgE (mouse monoclonal antibodies) Mouse monoclonal antibodies with 0.15% Kathon®CG covalently bound to a cellulose solid phase within a holder. Store at 2-8°C until the expiration.
9. UniCAP Specific IgE Calibrator Strip

Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

Product No. 10-9386-01, 5 strips per kit. 1 calibration curve per strip. Human IgE in buffer, Kathon CG 0.15%. Concentrations of 0.35 kU/L, 0.70 kU/L, 3.50 kU/L, 17.5 kU/L, 50.0 kU/L, 100.0 kU/L
Ready to use. Store at 2-8°C until the expiration date

10. UniCap Total IgE Calibrator Strip

Product No. 10-9387-01, 5 strips per kit, 1 calibration curve per strip. Human IgE in buffer, Kathon CG 0.15%. Concentrations 2; 10; 50; 200; 1000; 5000 kU/l. Ready to use. Store at 2-8°C until the expiration date.

11. UniCAP Specific IgE Curve Controls

Product No. 10-9312-01, 5 strips per kit. 3 CC-1 and 3 CC-2 on each strip. CC-1 = 0.70 kU/L, CC-2 = 17.5 kU/L. Human IgE in buffer. Kathon CG 0.15 %
Ready to use. Store at 2-8°C until the expiration date.

12. UniCap Total IgE Curve Controls

Product No. 10-9325-01, 5 strips per kit. 3 CC-1 and 3 CC-2 on each strip. CC-1 = 10 kU/l, CC-2 = 200 kU/l. Human IgE in buffer. Kathon CG 0.15 %
Ready to use. Store at 2-8°C until the expiration.

13. UniCAP Specific IgE Positive Control

Product No. 10-9449-01, 6 bottles x 4 determinations per bottle. Pooled human serum positive for specific IgE. Use Cat dander or e1 as High positive control and A.tennis or m6 as Medium positive control for each run. Positive control is ready to use. Store at 2-8°C until the expiration date. Precautions must be taken to avoid evaporation and contamination. Remove and recap the control vial from the instrument as soon as the pipetting of the samples is finished and serum incubation is started.

14. UniCAP Specific IgE Negative Control

Product No. 10-9445-01
6 bottles (4 determinations / bottle). Pooled human serum negative for all allergens. Use one negative control as Low control for each run. Negative control is ready to use. Store at 2-8°C until the expiration date. Precautions must be taken to avoid evaporation and contamination. Remove and recap the control vial from the instrument as soon as the pipetting of the samples is finished and serum incubation is started.

15. UniCAP Total IgE High, Medium and Low controls.

Product No. 10-9447-01

6 bottles (4 determinations / bottle). Pooled human serum supplied as High Total IgE control, Low Total IgE control and Medium Total IgE control (2 bottles of each control). Ready to use. Store at 2-8°C until the expiration date. Precautions must be taken to avoid evaporation and contamination. Remove and recap the control vial from the instrument as soon as the pipetting of the samples is finished and serum incubation is started.

16. Instrument sample pipette tips

Product number 12-3805-03, Aloka

17. Polypropylene test tube racks

18. Wooden sticks

19. MLA 200 µL pipette tips

20. Solid waste container bags Product No. 12-3807-25

21. Deionized water

22. Instrument Sample Rack

23. Measuring Cylinder

E. Instrumentation

1. Immunocap 1000, (Pharmacia Diagnostics, Kalamazoo, MI)

2. IDM (Immunocap Data Manager, Pharmacia Diagnostics, Kalamazoo, MI)

3. Maxi Mix II vortexer (Thermolyne)

4. MLA 200 and 50 µL pipettes

Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

7. Calibration and Calibration verification Procedures

A. Calibration of Instrument

Calibrators are provided in a strip with 6 positions, one for each calibrator point. A sealing foil covers the strip. The calibrators are pipetted with the sample pipette.

Calibration is performed every 28 days or when a new lot of conjugate is to be used or as indicated by curve control results. The calibration may need to be performed earlier than 28 days if the expected Quality control values show shifting. The calibration curve is set up for a specific lot number of conjugate by using six samples of known concentrations (calibrators), each sample run in duplicate. The curve is verified each day by running the curve controls. The patient results are calculated from this curve.

The operator must choose either curve controls or calibrators. Curve controls (CC-1 & CC-2) are run with every assay that does not have calibrators included. These controls are two points of known concentrations that are matched to an existing curve. There are two levels of limits for acceptance of curve controls, inner and outer limits, however for any curve control result that does not fall within the inner limits must have a new calibration curve run. The operator must choose either curve controls or calibrators.

To ensure the accuracy of test results, take the following steps for instrument maintenance:

1. Daily: Clean sample pipetting and loading areas with 70% ethanol. Run Curve Controls. Check in IDM for acceptability of curve control values.
2. Weekly: Perform Weekly Rinse followed by a reagent prime prior to next run.
3. Monthly: Clean all pipette and washing areas. Perform a system rinse followed by 2 reagent primes prior to next run. Run a new calibration curve and print out curve.
4. Semi-annually: Preventative maintenance through Phadia diagnostics or EMHC Biomedical Engineers.

B. Instructions for Calibration of Instrument

1. Remove calibrator strips from refrigerator and allow it to come to room temperature. Gently invert to mix.
2. In Load and Start field change status from Curve Control to Calibration.
3. Load calibrator strip in reagent tray.
4. Select OK.

8. Procedure Operating Instructions; Calculations; Interpretation of Results

A. Operating Procedure

Remove a sample box from -70 C freezer # 442 and keep it in refrigerator # 156 a day before testing to thaw at 2-8 C

1. Retrieve the samples to be tested from the refrigerator to bring to room temperature.
2. Switch ImmunoCAP Data Manager (IDM) computer on.
3. Switch ImmunoCAP 1000 Instrument system power on.
4. Perform daily maintenance.
5. Import test requests using the IDM.
6. Make a list of samples using consumable page in the IDM, make a tasklist, or use LIS pending log.
7. In the IDM, Request window, review the reagents needed for the assay run by highlighting all requests to be included in the assay run, then select "Menu" and "Consumables".
8. In the ISW, select "Utilities" and "Load Reagents"
9. Load necessary reagents by touching the appropriate area on the screen.
10. Pipette tips
11. Calibrator/Curve Control Strip

Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

12. Conjugate
13. Development
14. Stop Solution
15. Immunocap carrier
16. There must be full Washing Solution and Rinse Solution bottles loaded
17. Select "Load and Start"
18. Verify methods selected and status of Curve control or Calibration.
19. Select OK
20. The instrument will perform Assay initializing, assay priming, Blank measurement.
21. Load the QC rack
22. Vortex samples. Rim sample with a wooden stick if clot is present.
23. Load the Sample Racks.
24. Instrument will process Curve Controls/Calibration, QC and CDC Samples.
25. Confirm Calibration Curve or Curve Controls are OK and Quality Controls are within range.
26. Select "Samples Tab" to approve sample results.
27. After all sample pipetting is completed in Assay Processing press "End Assay"
28. Select Finish with Rinse and Shutdown. Instrument will complete testing, calculation of results, rinse and shut down.

B. Procedure following completion of testing.

1. Print laboratory report including Calibration information (if performed), Quality control information, Sample results, Lot # information (if selected), errors, and comments.
2. Return samples to their designated place in the box.
3. Return the sample box to -70°C freezer # 599, log on the freezer chart, when testing is completed on all the samples in the box.

C. Calculations

The IDM software uses Rodbard 5 parameter calculations to graph the calibrator concentrations as a loglog graph. The software then plots the sample response on the calibration curve to calculate the concentration into kU/L, or kU/L.

D. Special Procedure Notes – CDC Modifications

Not Applicable

9. Reportable Range of Results

A. Specific IgE

Measuring range for specific IgE for an undiluted sample is 0.35 – 100 kU/L.

Reportable range undiluted sample is <0.35 kU/L - 100 kU/L. Dilution of sample is required for determination of values higher than 100 kU/L IgE. Values >100 kU/L are verified by re-assay after the sample has been diluted 1:5 with sample diluent. If the repeat value is still >100 kU/L the sample may be diluted further to obtain the end point value up to 1,000 kU/L

B. Total IgE

Measuring range for Total IgE for an undiluted sample is 2 – 5000 kU/L

Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

Reportable range undiluted sample is <2.00 kU/l – 5000 kU/L. Dilution of sample is required for determination of values higher than 5000 kU/L. Values >5000 kU/L are verified by re-assay after the sample has been diluted 1:5 with sample diluent. If the repeat value is still >5000 kU/l the sample may be diluted further to obtain the end point value up to 50,000 kU/L.

10 Quality Control (QC) Procedures

A. Blind Quality Controls

Blind QC specimens are inserted by CDCNHANES prior to the arrival of the samples at the lab. These samples are prepared so as to emulate the patient samples; the labels used are identical to those used for patient samples with unique sample numbers. These controls are not identifiable by the EMHC technologists. Tests are performed and reported as sample person.

B. Bench Quality Controls

Positive Controls:

Because of reliability and availability, 2 controls of Specific IgE and 3 Total IgE controls from Pharmacia Diagnostics are currently used. For Specific IgE Positive control, e1 Cat dander and Positive control, m6 *Alternaria alternata* (A. tenuis) are used. This control is prepared from selected pooled human sera and contains IgE antibodies to e1 and m6 allergens. Positive control is ready to use. Store at 2-8 degrees and is good until the expiration date written on the control vial.

Negative control: e1 Cat dander is in use. For Total IgE, High, Low and Medium quality controls are in use. These controls are prepared and performed in the same manner as patient samples and analyzed as part of each run.

The following “Westgard rules” used for acceptability of the quality controls.

“13s refers to a control rule that is commonly used with a Levey-Jenning chart when the control limits are set as the mean plus 3s and the mean minus 3s. A run is rejected when a single control measured exceeds the mean plus 3s or the mean minus 3s control limit.”

The QC results are checked after QC is completed prior to the end of the run. The system is declared “in control” if all the QC results are within 3 SD reference range and the run is accepted. Establish the in-house reference ranges for QC by utilizing 20 QC values performed on different days on different calibration curves. Follow manufacturer’s recommendations of 3SD to calculate the range. According to Pharmacia Doc ID 353732 “Routine Clinical Immunoassays as e.g TSH usually has very low % CV. However, when measuring allergen-specific IgE antibodies, higher % CV are always obtained (c.f. CAP surveys etc). Over a long term testing including different lots of reagents, a total variation of about 8-12 % CV is to be expected for ImmunoCAP Specific IgE results, which for allergy testing is a very low figure and is much better than obtained with our competitors in allergy diagnostics.

The 95% confidence limit, +/- 2SD, means that 5% of the results are expected to be outside the ranges.

The 99% confidence limit, +/- 3SD, means that 1% of the results are expected to be outside the ranges.

Thus using +/- 2SD as acceptance range, 1 out of 20 runs will randomly not be accepted although there is nothing wrong with the assay run.

Using +/- 3SD as acceptance range only 1 out of 100 runs will randomly not be accepted although there is nothing wrong with the assay run.

This is the reason for our recommendation to use +/- 3SD for acceptance instead of +/- 2SD, and results between +/- 2SD and +/- 3SD should act as a “Warning signal” and check of instruments and routines should be made.

When necessary, ranges are updated to include more QC values generated from subsequent runs. If one of the three QC results is outside the 3 SD limits then repeat the control, which is out of range.

Record daily Quality Control results manually on an Excel worksheet. Record 0.34 any QC results generated as <0.35 from IC 1000.

C. External Quality Controls.

1. CAP Surveys:

Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

CAP surveys SE (A, B, C) are performed 3 times a year. For each survey six samples are tested for both Total IgE and 3-5 different specific allergens. For specific IgE only allergens are performed which are offered by the laboratory.

2. Quality Club:

Quality Club materials (12 vials) are received quarterly from Pharmacia. Total IgE on two samples and Specific IgE on one sample with 1-3 specific allergens are performed each month. Results of Quality Club are faxed to Sweden through Pharmacia Diagnostics. Sweden evaluates the results and sends the monthly and annual reports.

Note: According to Pharmacia technical support, most of the times the quality club results obtained from IC-1000 will have higher values for Total IgE and Specific IgE compared to UniCAP 100 and ImmunoCAP-250. The majority of the participants of the Quality Club are either UniCAP 100 or ImmunoCAP 250 users.

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

Pharmacia IDM will automatically edit if one calibration response unit is out of limit.

If the Curve controls are flagged as "not OK", recalibrate the instrument and read the same response units of QC and patient on the new calibrator curve. Verify the patient results, if controls are within range. Rerun the QC, if it is still out of range.

If calibration fails, call Pharmacia Technical support; check for the instrument errors and review patient raw data units. Repeat patients only if an instrument problem is discovered or if the run is ended prior to resolving the QC issue.

The variation in the calibration curve RESP is normal, as well as the quality control data generated. Several factors influence the RESP of the quality control and the calibrators:

1. Conjugate lot #.
2. ImmunoCAP lot #
3. Temperature and humidity changes in the laboratory.

The minimum and maximum ranges should be wide enough to represent the variation in data from all variables involved. It is important to establish QC ranges using more than one calibration curve. Since the precision of the IC1000 is very good, the data points generated from using only one calibration curve will be too tight. Pharmacia's CoE has also stated that expected CV % long term for quality control may be up to 15 % CV. (refer to the letter from Pharmacia Diagnostics)

Acceptance Rules

Calibration curve

In ImmunoCAP 1000 numerous functions are checked before the analytical run is considered by the software as accepted or not. If all of required functions have performed within the specific limits, the analytical run is accepted by the software and thus accepted by the operator.

- a. Self-check routines
Before each assay run the instrument will automatically perform a self-check of important functions such as the assay blank.
- b. Validity of Calibration Curve
ImmunoCAP 1000 will automatically verify the calibration curve or the curve controls are within the preprogrammed limits and give a recommendation to accept or not accept the validity of calibration curve.
- c. Acceptance of Calibration Curve
Signal levels obtained from runs with calibrator points are subject to a curve fitting procedure including examination of the obtained signal values against predetermined expected levels and limits.

When all calibrator replicates fall within expected limits the calibration curve is evaluated, accepted as valid, stored and set as active.

When one replicate is discarded, the calibration curve will be evaluated, used and set as active.

If all replicates of the lowest calibration point are discarded- the calibration curve will not be evaluated and not set as active or two or more replicates are discarded, run a new calibrator curve and evaluate the responses on the new valid curve.

Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

- d. Acceptance of Analytical runs
Assay runs with newly established and valid calibration curves are considered as accepted by the software. Together with other assay performance parameters, such as quality controls within limits, the included analytical runs are recommended by the software to be accepted by the operator.

In assay runs with stored calibration curves, curve controls can be used. If obtained values for the curve controls are within the preprogrammed limits, the calibration curve validity is verified and the assay run is considered as accepted by the software. Together with other assay performance parameters, such as quality controls within limits, the included analytical runs are recommended by the software to be accepted by the operator.

12. Limitations of Method; Interfering Substances and Conditions

- A. Studies show no interference from icteric, lipemic or hemolytic samples. These studies are documented on the web site www.unicapinvitrosight.com (Look under Methods, Specific IgE, and Sample Collection).
- B. The cross reactivity with other human immunoglobulins is nonexistent at physiological concentrations of IgA, IgD, IgM, and IgG.
- C. A definitive clinical diagnosis should not be based on the results of any single diagnostic method, and should only be made by the physician after all clinical and laboratory findings have been evaluated.
- D. Allergen specific IgE antibody levels as measured by in vitro assays are sometimes used as grounds for instituting immunotherapy, however, the results of a specific IgE test should not be the only consideration when selecting an initial dose for immunotherapy. Prior to implementing immunotherapy, a skin test with a planned initial dilution of the immunotherapy solution should be performed to prove that the patient tolerates in vivo administration of this allergenic extract.
- E. In food allergy, circulating IgE antibodies may remain undetectable despite a convincing clinical history because these antibodies may be directed towards allergens that are revealed or altered during industrial processing, cooking, or digestion and therefore do not exist in the original food for which the patient is tested.
- F. False positive test results in persons who are tested for food allergies may lead to inappropriate dietary restrictions while false negative results in food sensitive persons may result in anaphylactic reactions of varying severity.
- G. Identical results for different allergens may not be associated with clinically equivalent manifestations, due to differences in patient sensitivities.
- H. Latex specific IgE antibodies may show cross reactivity with ragweed and certain food allergens such as banana, avocado, kiwi, and chestnut. Clinical diagnosis should be made by a physician after all clinical and laboratory findings have been evaluated and not solely on the results of an in vitro diagnostic test.

13. Reference Ranges (Normal Values)

Manufacturer reference ranges for Total IgE were validated. Samples were randomly collected from 45 EMHC employees (male and female) and tested for Total IgE. The mean value and CV and 2 SD was calculated. Current proposed normal ranges for serum specific IgE is <0.35 kU/L, for both sexes and all ages. Current adult normal range for Total IgE is 0 kU/L – 119 kU/L for both sexes.

According to the manufacturer when samples from healthy non-allergic blood donors were tested against the existing panel of ImmunoCAP Specific IgE allergens, the response units were well below the 0.35 kU/L calibrator. Values of 0.35 kU/L or greater, where A represents allergen-specific antibodies, and above represent a progressive increase in the relative concentration of allergen-specific antibodies. Results below 0.35 kU/L represent absent or undetectable levels of allergen-specific antibodies.

According to a manufacturer study using healthy children, serum total IgE values may vary up to 10 years of age. After the peak at the age of 10 years, serum total IgE values decline to adult values.

14. Critical Call Results (“Panic Values”)

Not applicable for this procedure.

Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

15. Specimen Storage and Handling During Testing

Allow the samples to thaw in the refrigerator at 2-8° C temperature. Bring the number of samples to be tested to room temperature. Maintain the samples at room temperature during the testing process. Store the sample box back in the refrigerator at 2-8 until the testing is completed on all the samples in the box.

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

There are no acceptable alternative methods of analysis for Specific and Total IgE. Specimens are stored at -70°C for testing when system is functional.

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

Print results using the ImmunoCAP Data Manager (IDM) Software, write run number, date and Initial. Export the data from IDM to LIS using the interface. Post the results in LIS. Review the results for abnormal values, unusual and exceptional values. Do not verify any result if the values are > 100 for specific Ige and > 5000 for Total IgE. Verify results in LIS if all results appear ok. File the print outs and the QC prints in the appropriate folders. For CDC specimens enter "SC" \ F5 to enter the appropriate code number where results are not available, for example specimen QNS for testing enter **21**; QNS for repeat testing enter **22**. Do not report any results >100 for Specific IgE and / or >5,000 for Total IgE. Make dilutions of samples with specific results >100 and total results >5,000 using sample Diluent. Perform the specific or total testing only for the allergen with >100 or >5,000 previous result. For CDC samples use the code # 103 for reporting If results are > 1,000 for specific IgE and > 50,000 for total IgE.

This study does not have any reportable critical results assigned.
All data is reported weekly by e-mail to Westat ISIS computer system.

Sensitivity

The detection limit is <0.35 kU/L for Specific IgE.
The detection limit is <2 kU/L for Total IgE

Specificity

The crossreactivity with other human immunoglobulins is non-detectable at physiological concentrations of IgA, IgD, IgM and IgG.

Recovery

Mean recovery is 98% for Total IgE.

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

A. Procedure – Triaging

1. Record Date/Time on shipping label and CDC log. Record Stand # on CDC log.
2. Take out Manifest, FedEx return label and box(s).
3. Record Manifest Number (Shipment ID) on CDC log. Keep paperwork to file.
4. Record Box ID and specimen conditions as Frozen, Slushy, Cold or Ambient on CDC log.
5. Record the number of tubes in the box and printed on the manifest on the CDC log. (If there is a discrepancy of specimen number then contact a manger who will e-mail problem to CDC.)
6. Record the ID of the first specimen and the last specimen on the CDC log.
7. File the Manifest and FedEx paperwork in CDC manual.
8. Use return FedEx label to ship packaging back to CDC.
9. Order samples in LIS as CDC1 or CDC2 as indicated on the manifest and record specimen ID number from first sample in the box on the CDC log. The ordering manifest is usually reformatted from the e-mailed manifest for ease of ordering. (If not ordering the same day, place the samples in -70 freezers # 442. Log on the excel sheet on the freezer.)

CDC1

Total IgE

Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

d1 = D. pteronyssinus (dust mite)
e1 = Cat epithelium and dander
e5 = Dog dander
i6 = German Cockroach
f13 = Peanut
f1 = Egg
f2 = Milk
m6 = Alternaria alternata
d2 = D. farinae (dust mite)

CDC2

Total IgE
d2 = D. farinae
d1 = D. pteronyssinus
e1 = Cat epithelium and dander
e5 = Dog Dander
i6 = German Cockroach
w1 = Common ragweed
g5 = Rye grass
m6 = Alternaria alternata
g2 = Bermuda grass
t7 = White oak
t3 = Birch tree
f13 = Peanut
f24 = Shrimp
f1 = Egg White
m3 = Aspergillus fumigatis
w11 = Russian thistle
e72 = Mouse urine protein
e74 = Rat urine protein
f2 = Milk

When ordering, if vial ID doesn't match manifest ID for given slot then find ID on manifest, order correct test. Place correct specimen collection information in SOFT.

If ID is not on the manifest, then inform a supervisor and continue ordering testing for rest of specimens.

After specimens are ordered place or replace them back in -70 freezers # 442 in same location. (If ordering completed same day as delivery, place the samples in -70 freezer # 442 and log all demanded information on the excel sheet on the freezer.)

Take out the sample box to be tested and bring it to room temperature. If testing is not completed on all the samples in the box or if there are samples selected for 2% repeats for the next run, store the box in refrigerator. Do not freeze and thaw. Store the completed samples in the original box and then log it on an Excel template into -70°C freezer #599.

19. Summary Statistics and QC Graphs

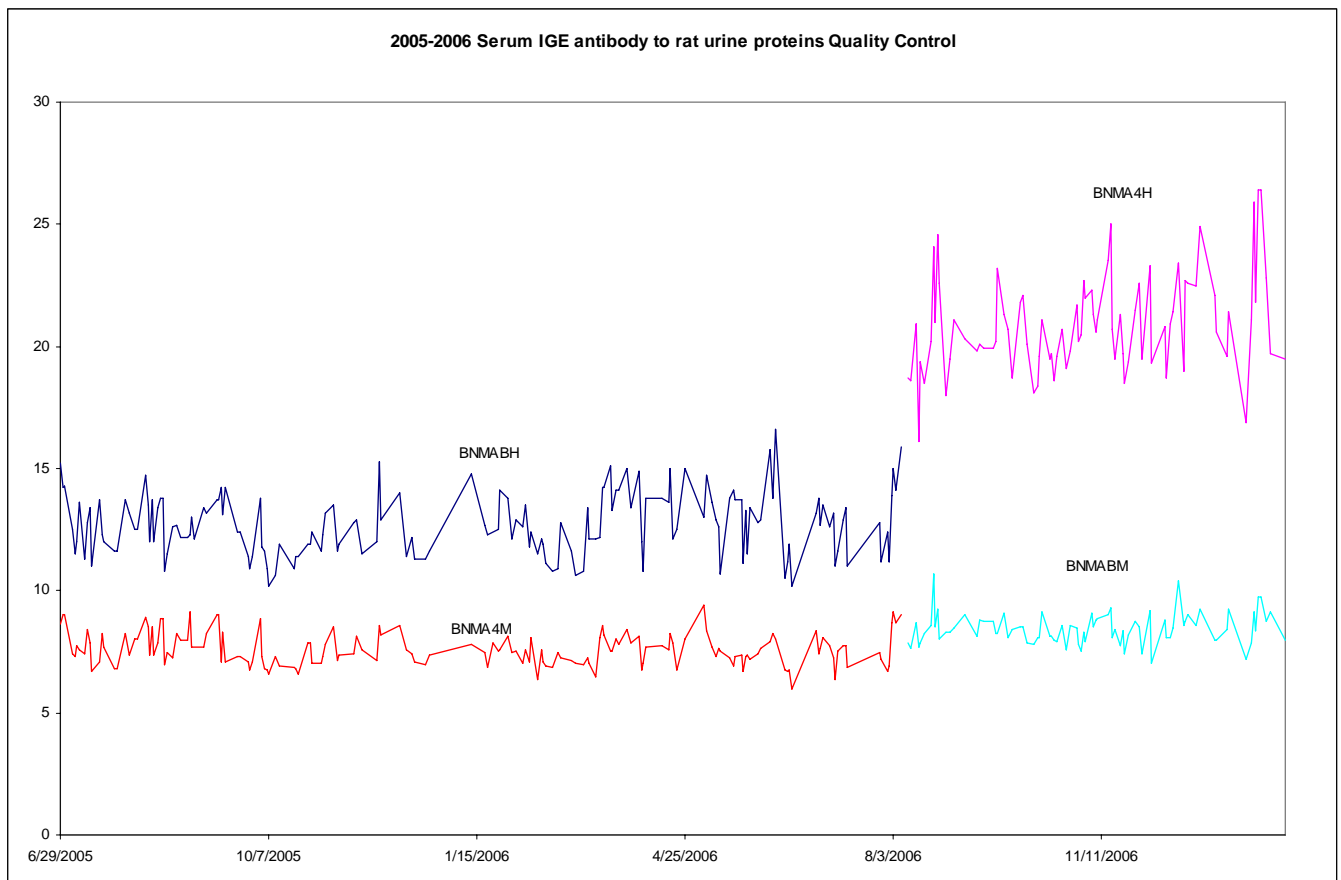
There is one set of controls for the total IgE and one set of controls for all allergen-specific IgE tests. The allergen-specific IgE controls test the Pharmacia system that employs several hundred different allergen-containing reagents. Each allergen-specific reagent was quality controlled by the manufacturer.

Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

A. Serum IGE antibody to rat urine proteins

Summary Statistics for Serum IGE antibody to rat urine proteins by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BL1AN	237	6/29/2005	12/28/2006	0.340	0.000	0.0
BNMA4	167	6/29/2005	8/7/2006	7.602	0.672	8.8
BNMA4	167	6/29/2005	8/7/2006	12.701	1.276	10.0
BNMAB	83	8/10/2006	2/7/2007	8.444	0.644	7.6
BNMAB	83	8/10/2006	2/7/2007	20.878	2.026	9.7
BL1AY	13	1/4/2007	2/7/2007	0.340	0.000	0.0

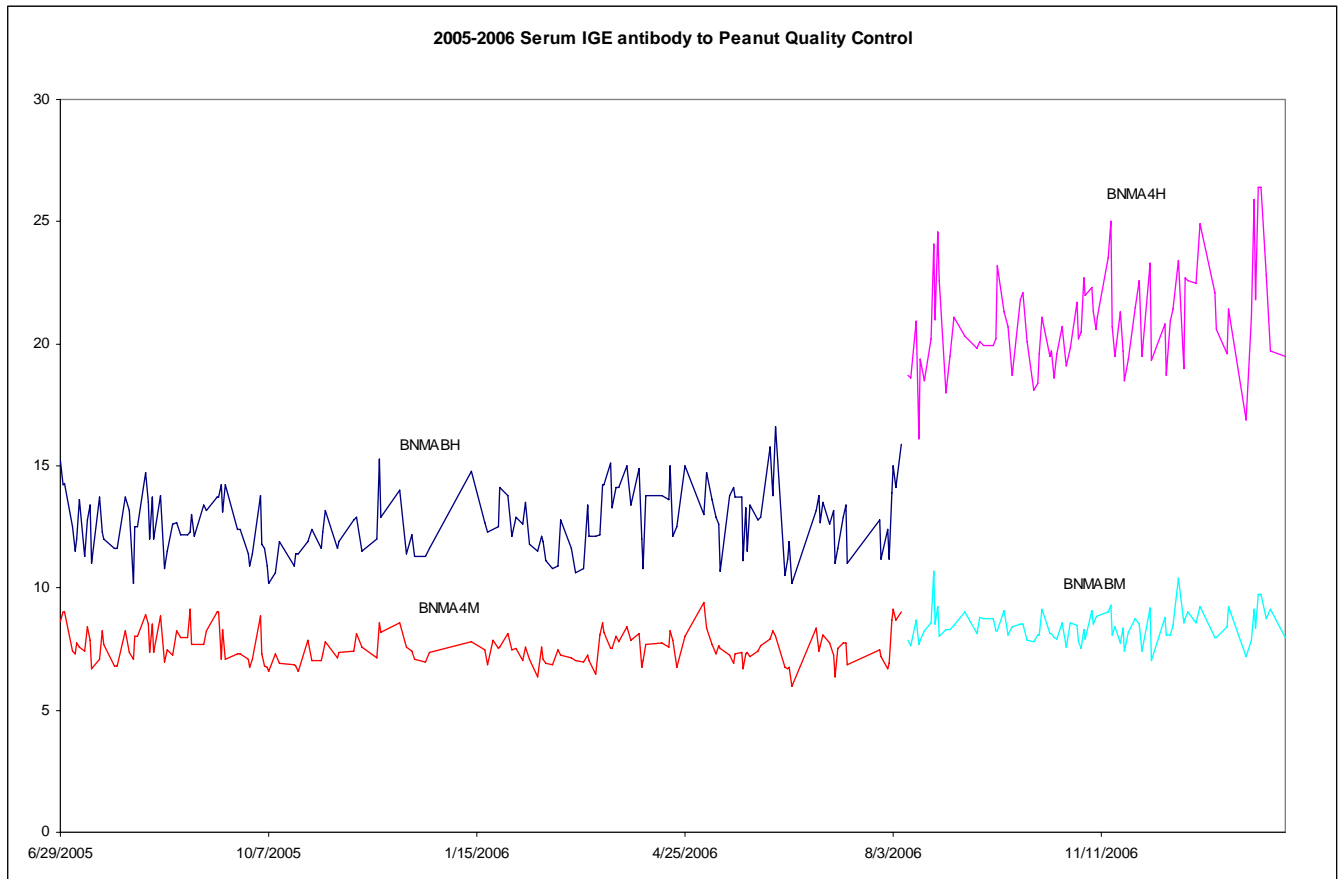


Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

B. Serum IGE antibody to Peanut

Summary Statistics for Serum IGE antibody to Peanut by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BL1AN	233	6/29/2005	12/28/2006	0.340	0.000	0.0
BNMA4	163	6/29/2005	8/7/2006	7.579	0.668	8.8
BNMA4	163	6/29/2005	8/7/2006	12.677	1.299	10.2
BNMAB	83	8/10/2006	2/7/2007	8.444	0.644	7.6
BNMAB	83	8/10/2006	2/7/2007	20.878	2.026	9.7
BL1AY	13	1/4/2007	2/7/2007	0.340	0.000	0.0

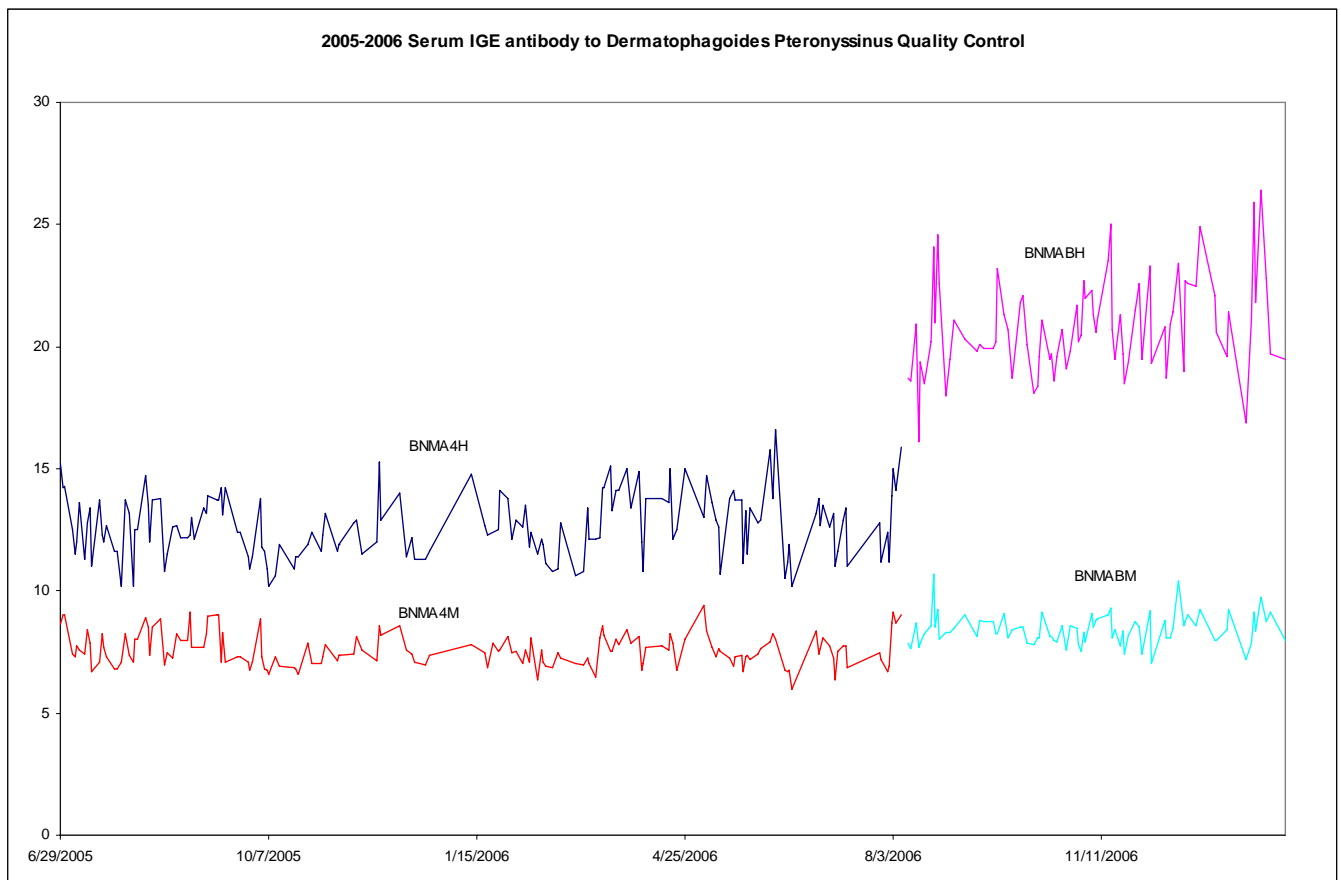


Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

C. Serum IGE antibody to Dermatophagoides Pteronyssinus

Summary Statistics for Serum IGE antibody to Dermatophagoides Pteronyssinus by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BL1AN	236	6/29/2005	12/28/2006	0.340	0.000	0.0
BNMA4	166	6/29/2005	8/7/2006	7.588	0.673	8.9
BNMA4	166	6/29/2005	8/7/2006	12.673	1.303	10.3
BNMAB	82	8/10/2006	2/7/2007	8.428	0.631	7.5
BNMAB	82	8/10/2006	2/7/2007	20.811	1.943	9.3
BL1AY	12	1/4/2007	2/7/2007	0.340	0.000	0.0

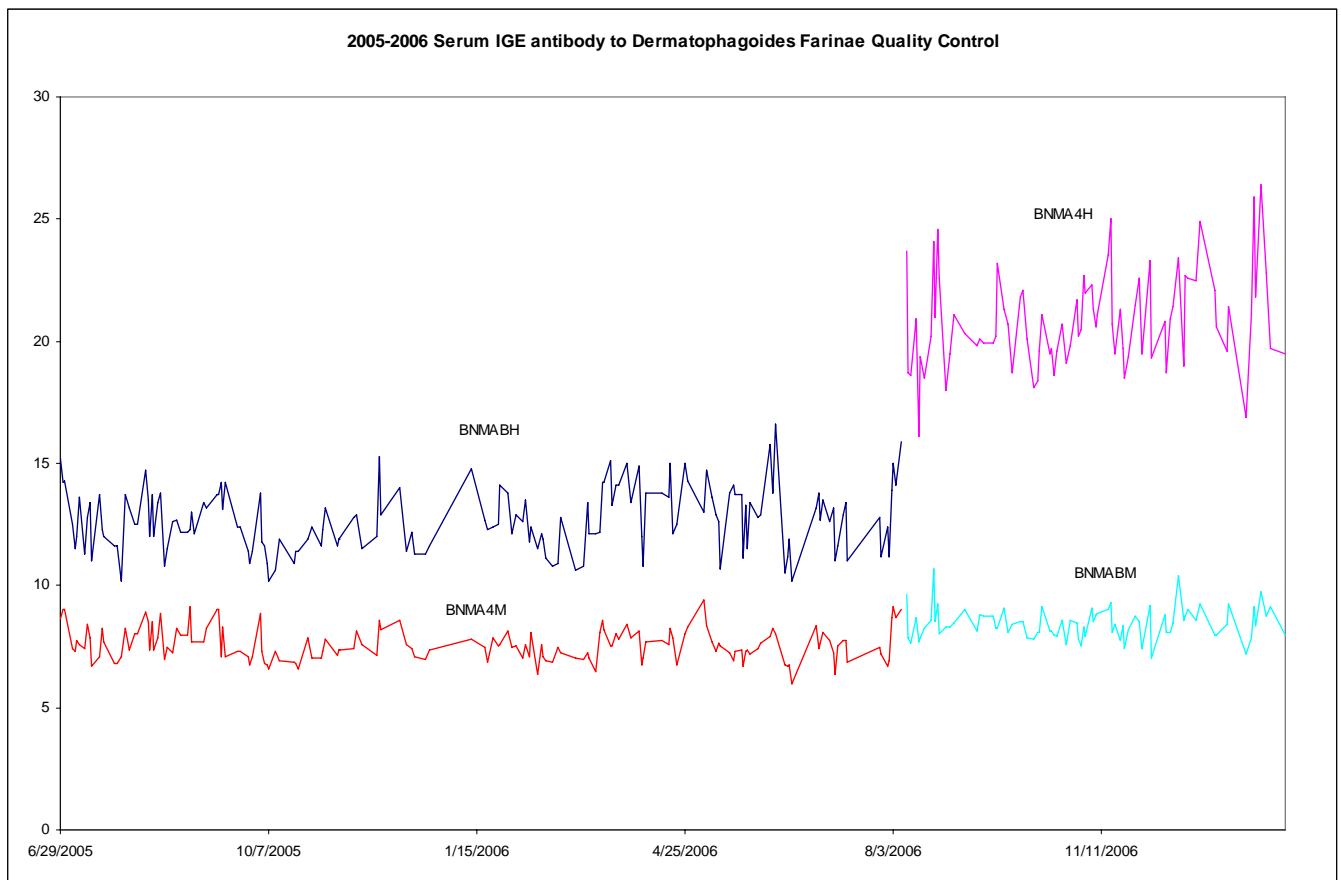


**Specific IgE/Total IgE Allergens in Serum
NHANES 2005-2006**

D. Serum IGE antibody to Dermatophagoides Farinae

Summary Statistics for Serum IGE antibody to Dermatophagoides Farinae by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BL1AN	237	6/29/2005	12/28/2006	0.340	0.000	0.0
BNMA4	166	6/29/2005	8/7/2006	7.589	0.666	8.8
BNMA4	166	6/29/2005	8/7/2006	12.691	1.293	10.2
BNMAB	83	8/9/2006	2/7/2007	8.443	0.641	7.6
BNMAB	83	8/9/2006	2/7/2007	20.846	1.957	9.4
BL1AY	12	1/4/2007	2/7/2007	0.340	0.000	0.0

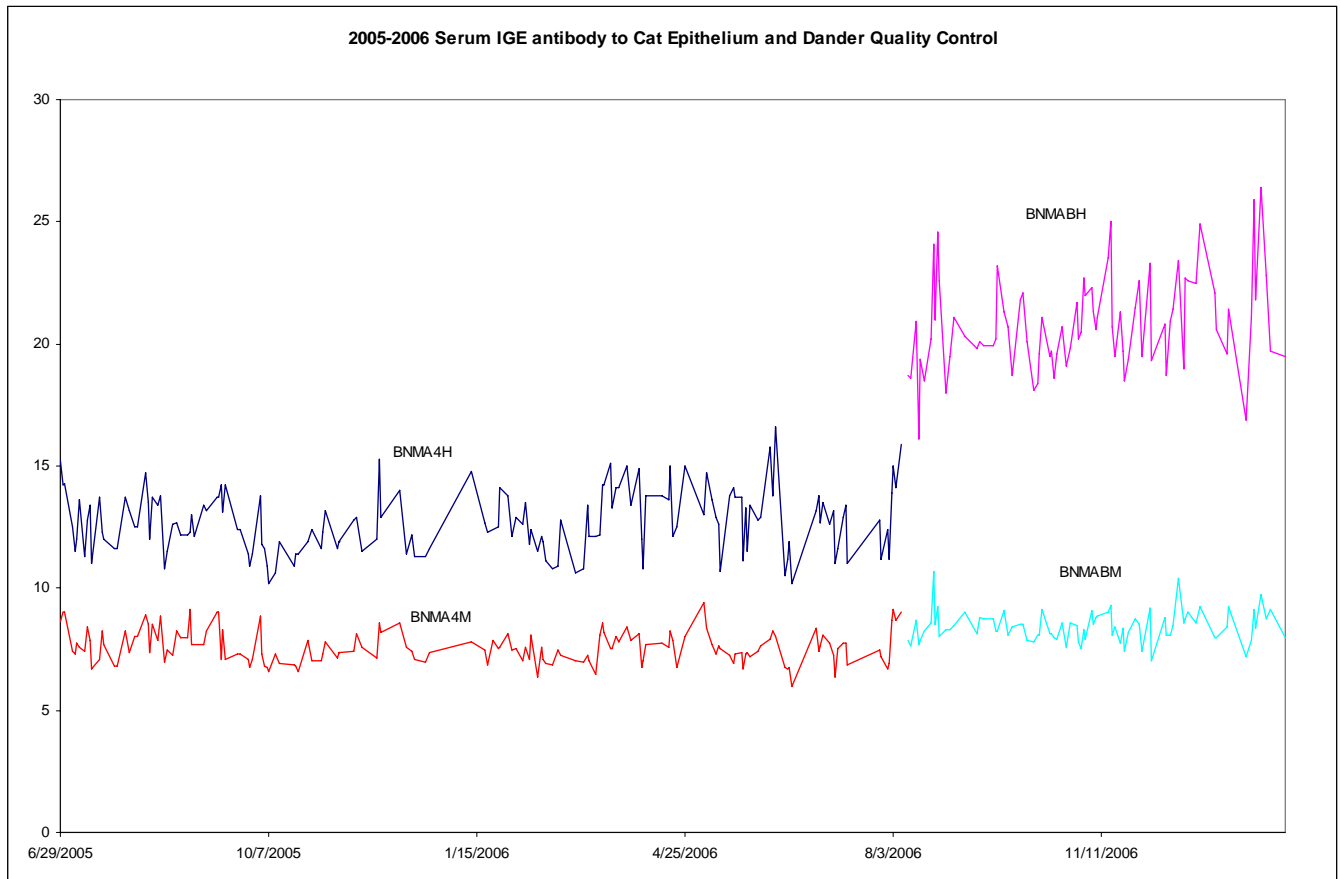


**Specific IgE/Total IgE Allergens in Serum
NHANES 2005-2006**

E. Serum IGE antibody to Cat Epithelium and Dander

Summary Statistics for Serum IGE antibody to Cat Epithelium and Dander by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BL1AN	233	6/29/2005	12/28/2006	0.340	0.000	0.0
BNMA4	163	6/29/2005	8/7/2006	7.589	0.668	8.8
BNMA4	163	6/29/2005	8/7/2006	12.701	1.283	10.1
BNMAB	82	8/10/2006	2/7/2007	8.428	0.631	7.5
BNMAB	82	8/10/2006	2/7/2007	20.811	1.943	9.3
BL1AY	12	1/4/2007	2/7/2007	0.340	0.000	0.0

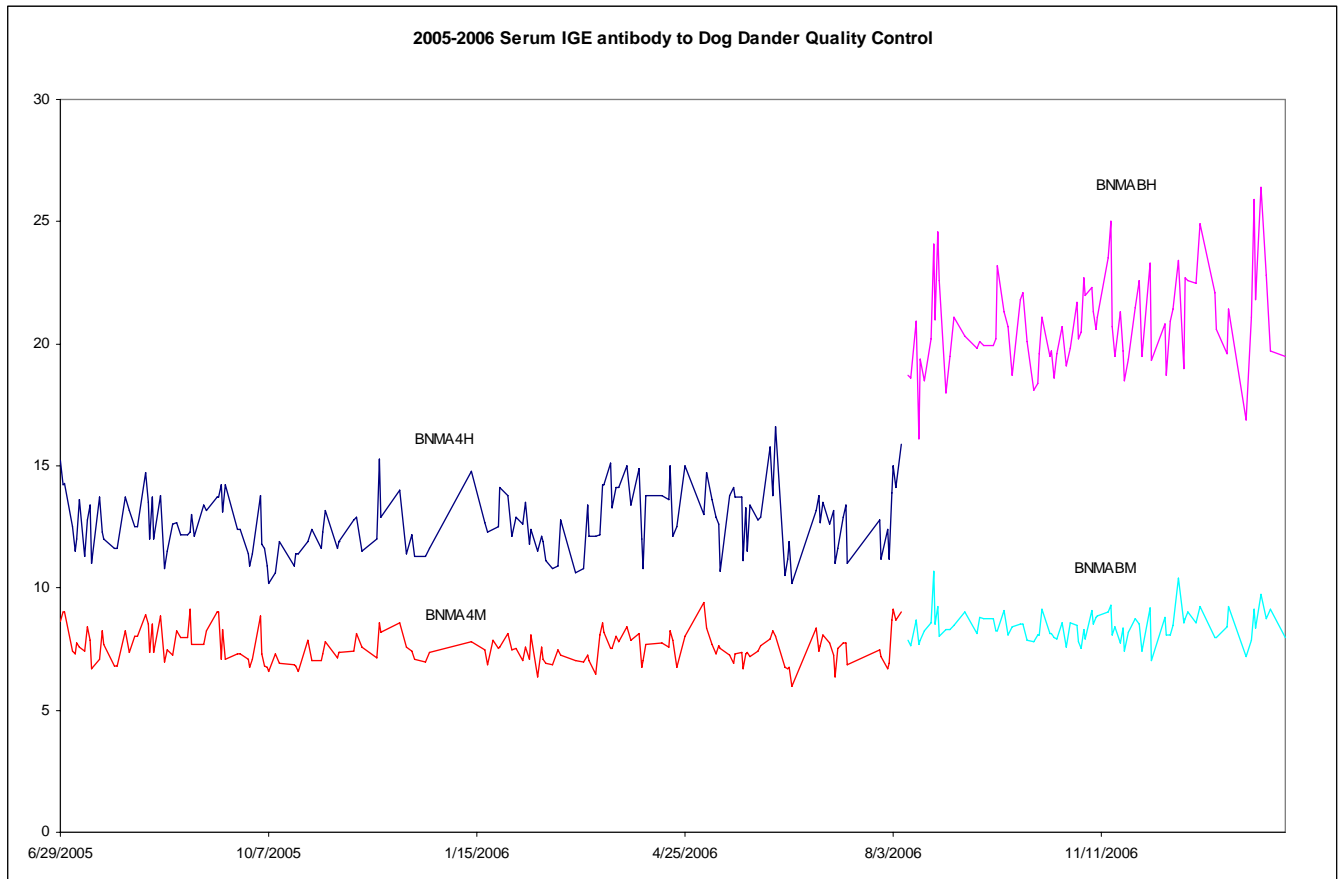


**Specific IgE/Total IgE Allergens in Serum
NHANES 2005-2006**

F. Serum IGE antibody to Dog Dander

Summary Statistics for Serum IGE antibody to Dog Dander by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BL1AN	233	6/29/2005	12/28/2006	0.340	0.000	0.0
BNMA4	163	6/29/2005	8/7/2006	7.586	0.668	8.8
BNMA4	163	6/29/2005	8/7/2006	12.692	1.283	10.1
BNMAB	82	8/10/2006	2/7/2007	8.428	0.631	7.5
BNMAB	82	8/10/2006	2/7/2007	20.811	1.943	9.3
BL1AY	12	1/4/2007	2/7/2007	0.340	0.000	0.0

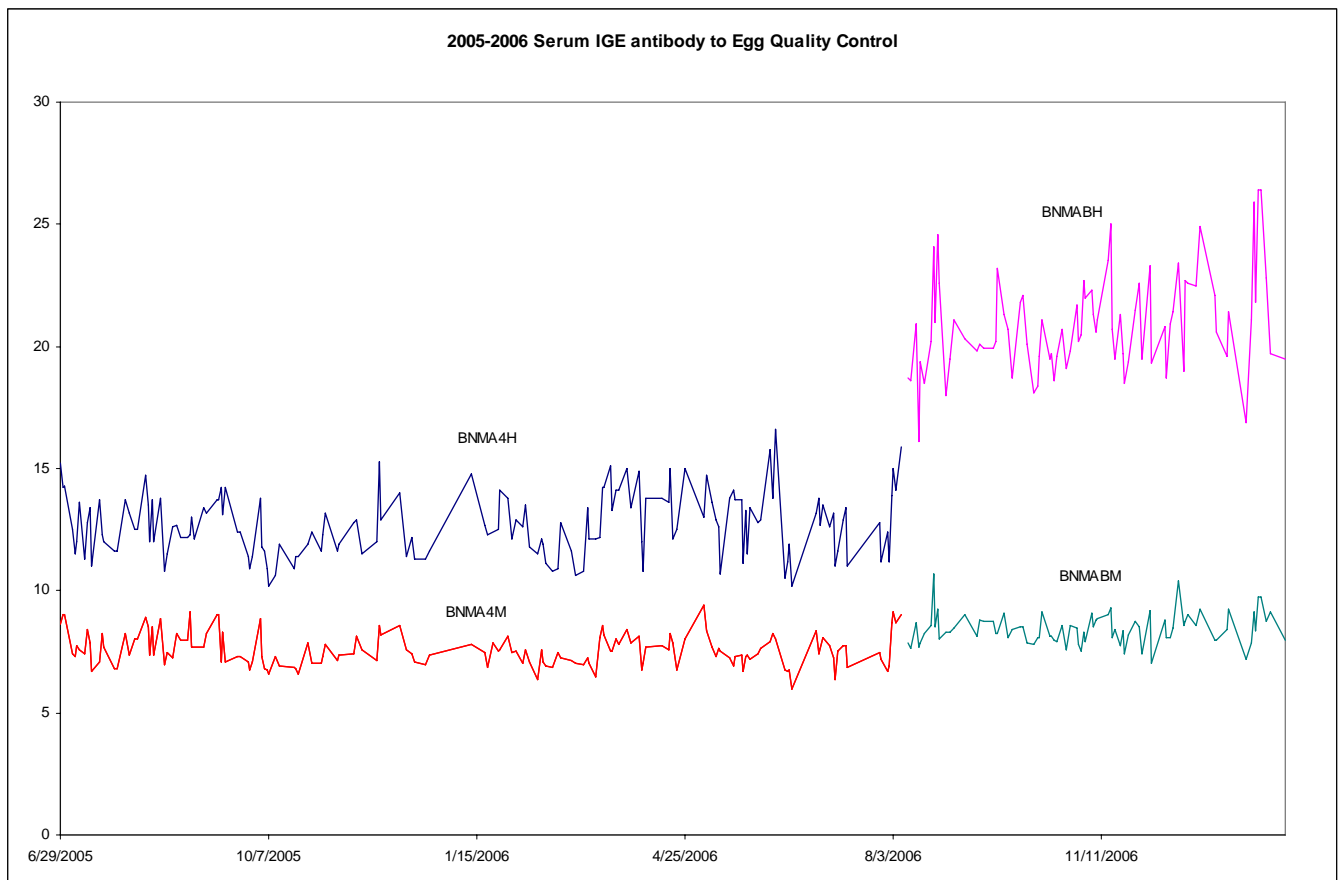


**Specific IgE/Total IgE Allergens in Serum
NHANES 2005-2006**

G. Serum IGE antibody to Egg

Summary Statistics for Serum IGE antibody to Egg by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BL1AN	232	6/29/2005	12/28/2006	0.340	0.000	0.0
BNMA4	162	6/29/2005	8/7/2006	7.582	0.669	8.8
BNMA4	162	6/29/2005	8/7/2006	12.692	1.288	10.1
BNMAB	83	8/10/2006	2/7/2007	8.444	0.644	7.6
BNMAB	83	8/10/2006	2/7/2007	20.878	2.026	9.7
BL1AY	13	1/4/2007	2/7/2007	0.340	0.000	0.0

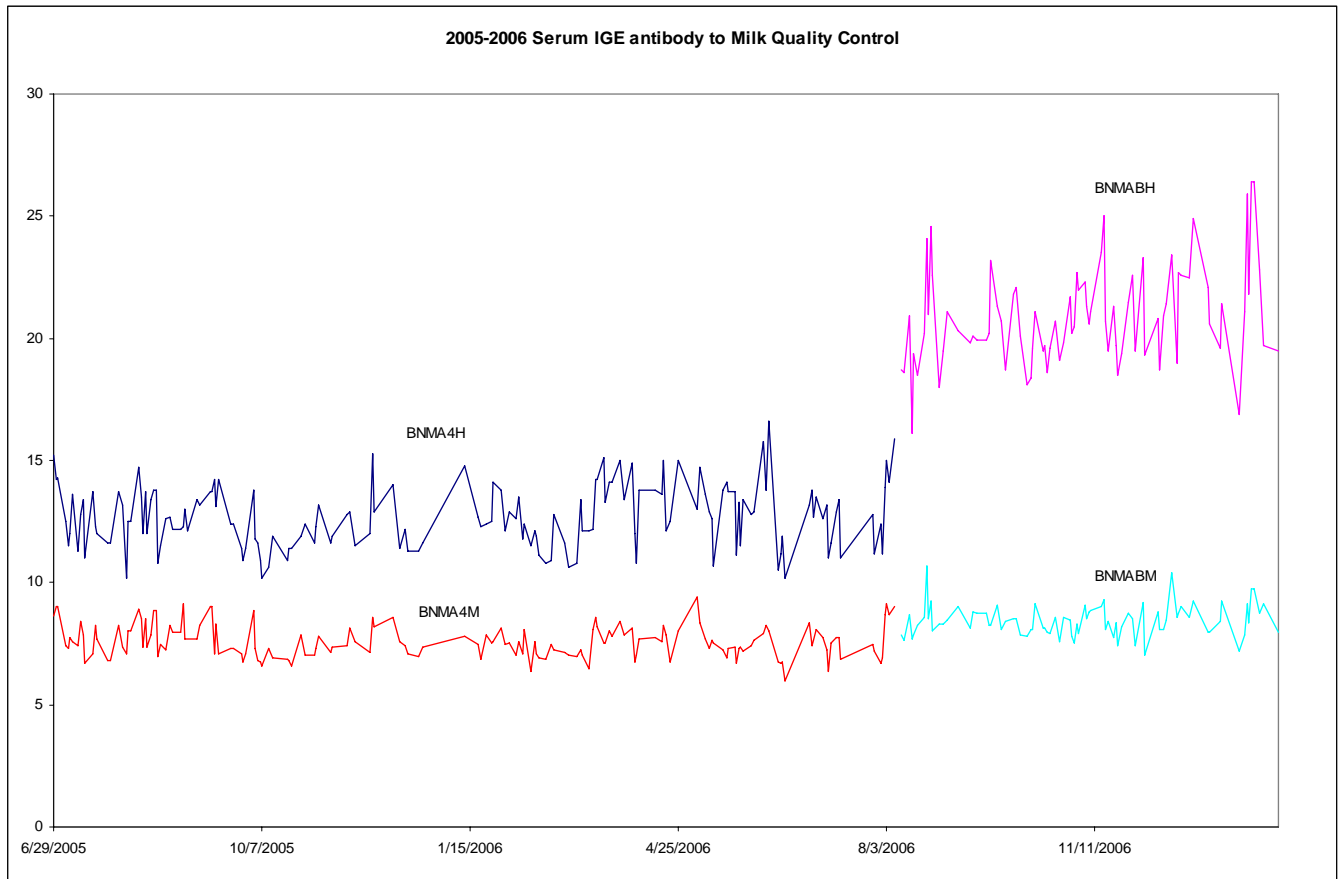


Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

H. Serum IGE antibody to Milk

Summary Statistics for Serum IGE antibody to Milk by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BL1AN	237	6/29/2005	12/28/2006	0.340	0.000	0.0
BNMA4	167	6/29/2005	8/7/2006	7.594	0.670	8.8
BNMA4	167	6/29/2005	8/7/2006	12.683	1.288	10.2
BNMAB	83	8/10/2006	2/7/2007	8.444	0.644	7.6
BNMAB	83	8/10/2006	2/7/2007	20.878	2.026	9.7
BL1AY	13	1/4/2007	2/7/2007	0.340	0.000	0.0

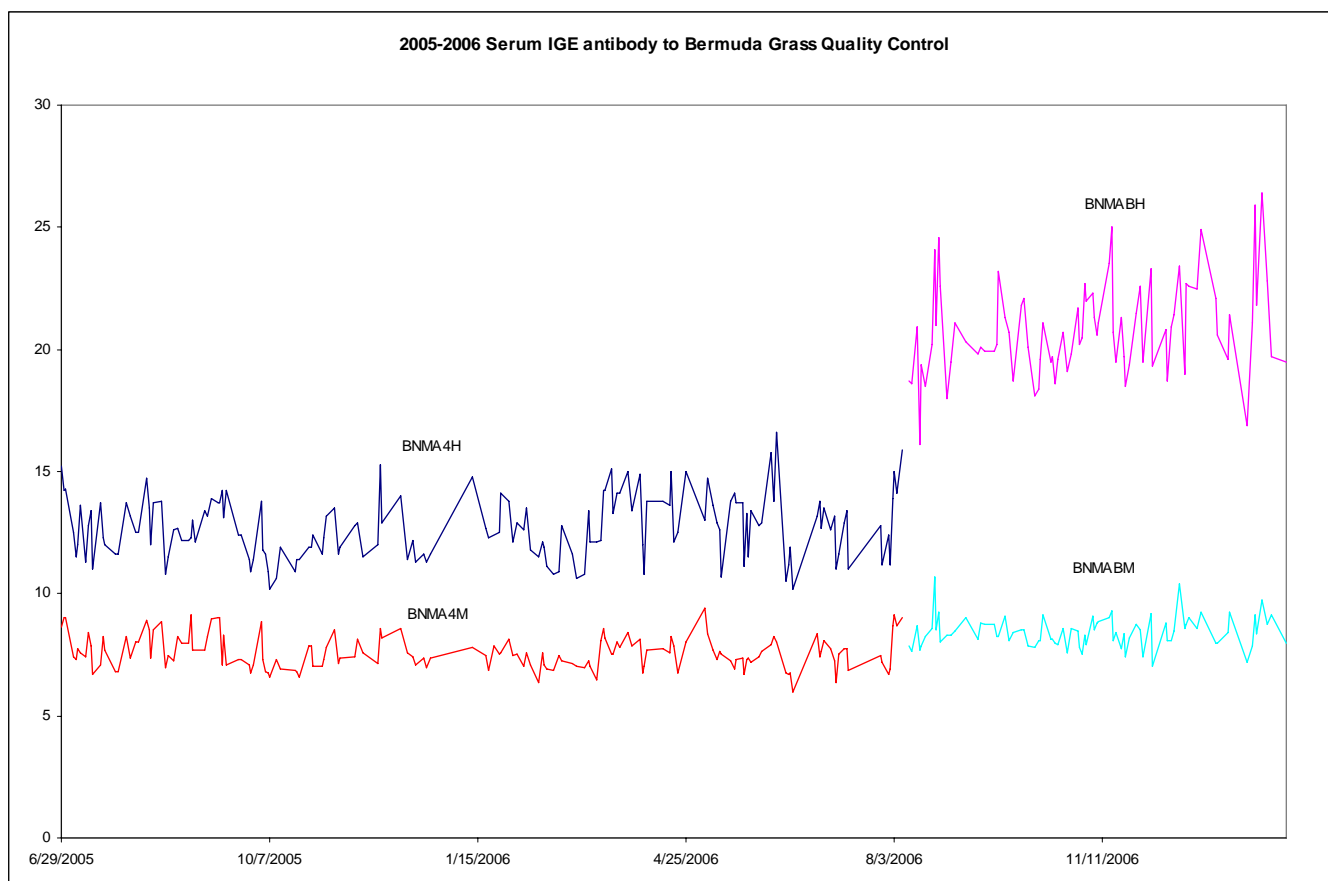


Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

I. Serum IGE antibody to Bermuda Grass

Summary Statistics for Serum IGE antibody to Bermuda Grass by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BL1AN	235	6/29/2005	12/28/2006	0.340	0.000	0.0
BNMA4	165	6/29/2005	8/7/2006	7.598	0.676	8.9
BNMA4	165	6/29/2005	8/7/2006	12.697	1.284	10.1
BNMAB	82	8/10/2006	2/7/2007	8.428	0.631	7.5
BNMAB	82	8/10/2006	2/7/2007	20.811	1.943	9.3
BL1AY	12	1/4/2007	2/7/2007	0.340	0.000	0.0

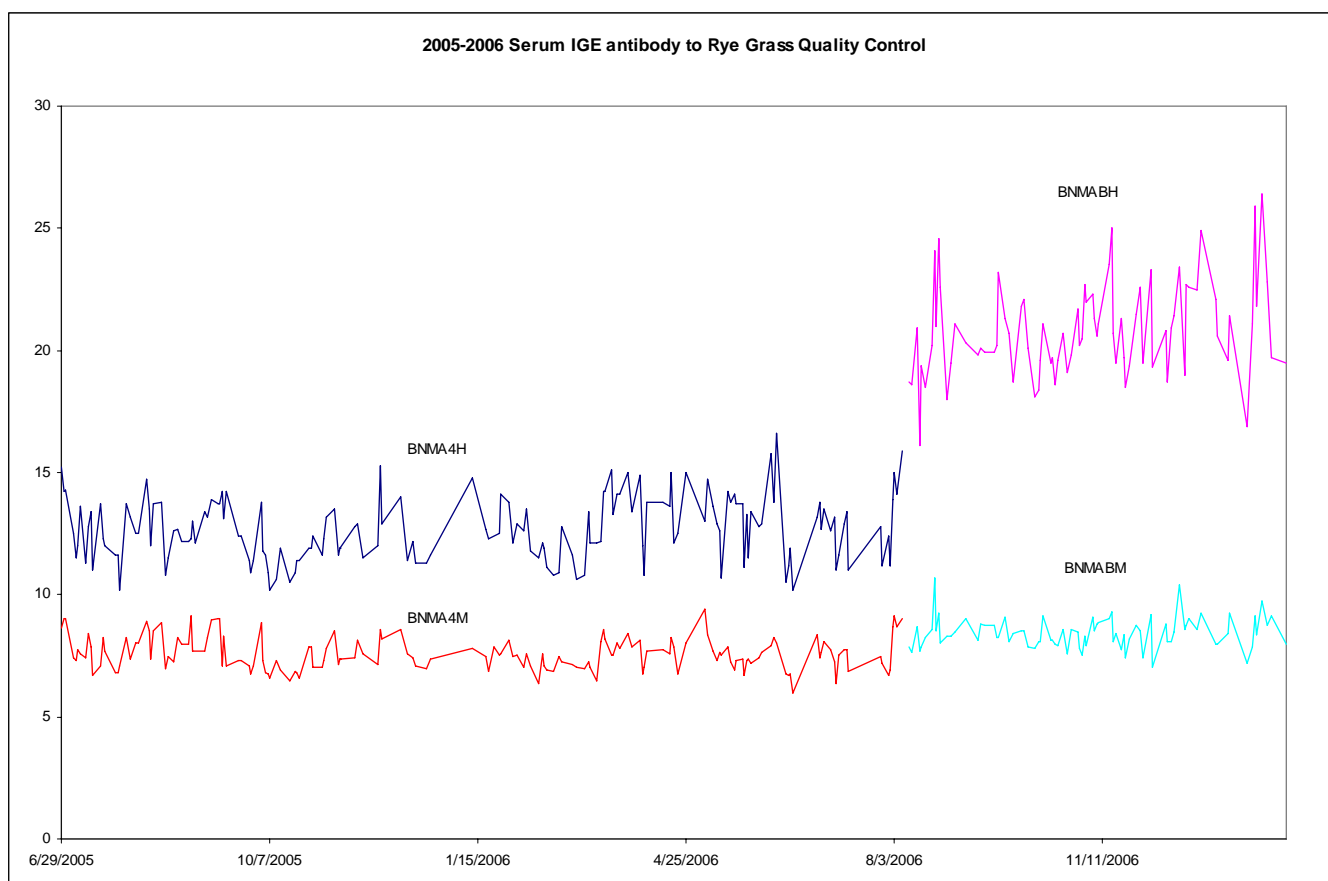


Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

J. Serum IGE antibody to Rye Grass

Summary Statistics for Serum IGE antibody to Rye Grass by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BL1AN	238	6/29/2005	12/28/2006	0.340	0.000	0.0
BNMA4	168	6/29/2005	8/7/2006	7.590	0.678	8.9
BNMA4	168	6/29/2005	8/7/2006	12.680	1.302	10.3
BNMAB	82	8/10/2006	2/7/2007	8.428	0.631	7.5
BNMAB	82	8/10/2006	2/7/2007	20.811	1.943	9.3
BL1AY	12	1/4/2007	2/7/2007	0.340	0.000	0.0

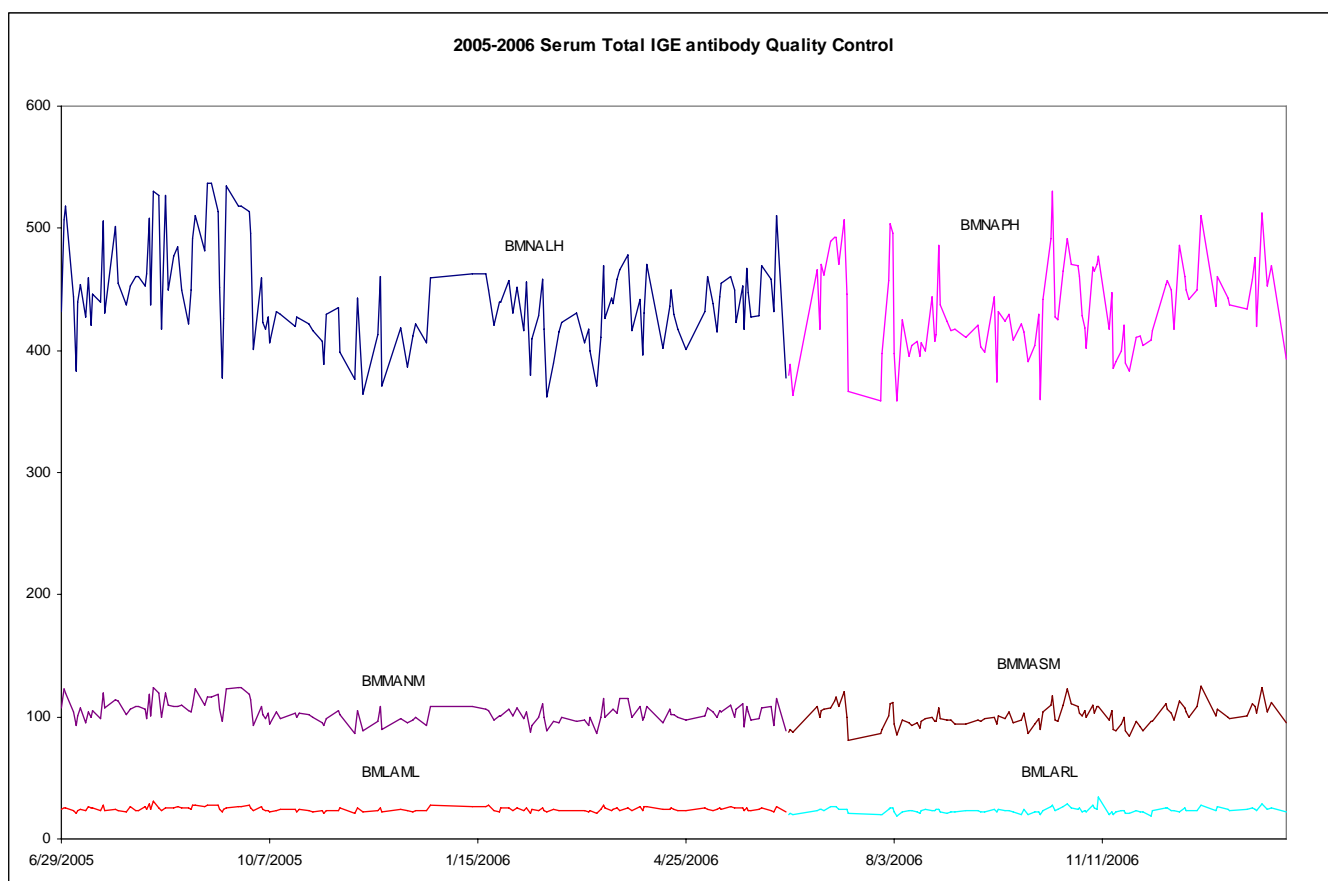


Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

K. Serum Total IGE antibody

Summary Statistics for Serum Total IGE antibody by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BMLAM	142	6/29/2005	6/12/2006	24.485	1.849	7.6
BMMAN	142	6/29/2005	6/12/2006	103.95	8.423	8.1
BMNAL	142	6/29/2005	6/12/2006	442.359	38.524	8.7
BMLAR	104	6/13/2006	2/7/2007	23.489	2.313	9.8
BMMAS	104	6/13/2006	2/7/2007	100.824	8.781	8.7
BMNAP	104	6/13/2006	2/7/2007	433.404	38.122	8.8

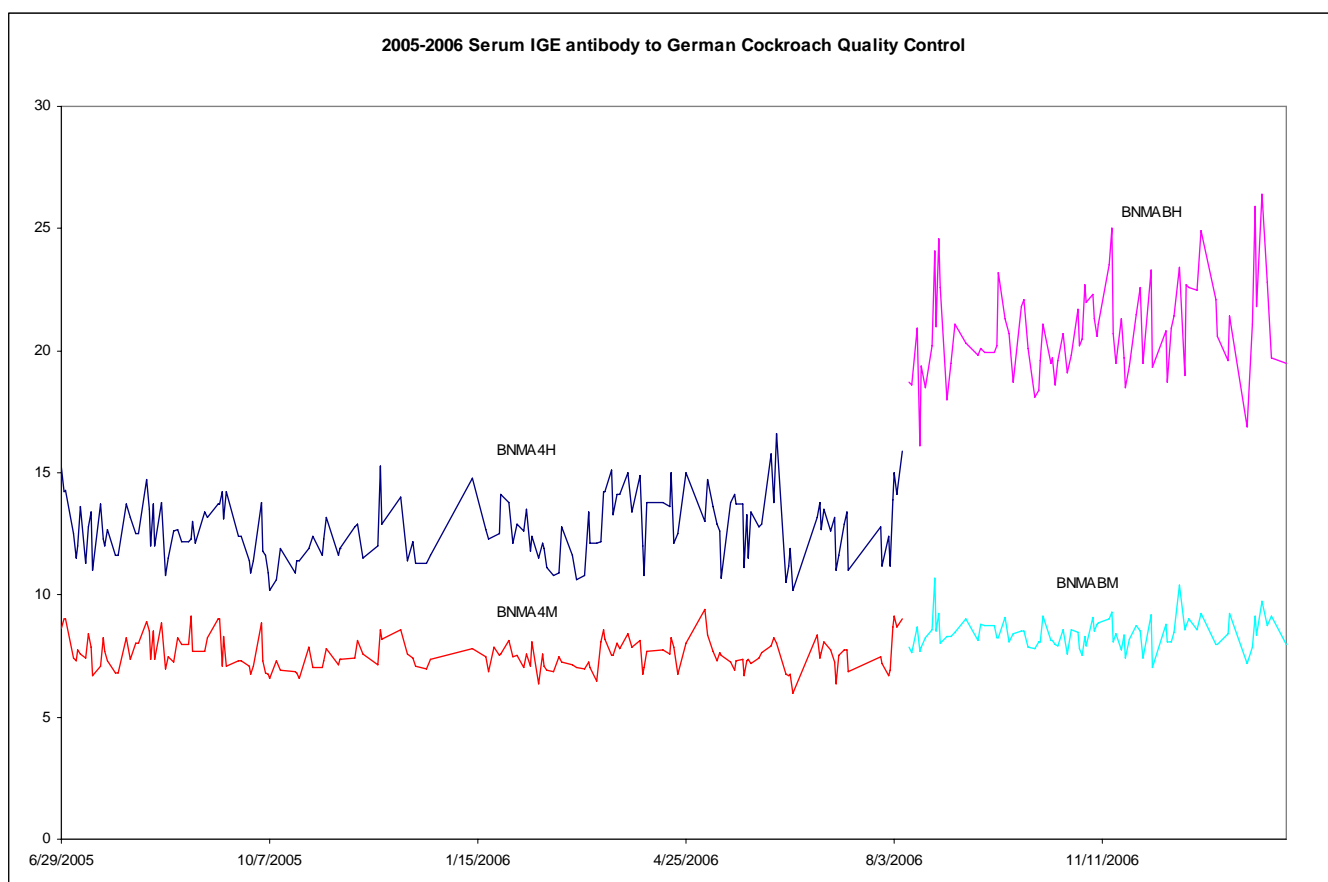


Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

L. Serum IGE antibody to German Cockroach

Summary Statistics for Serum IGE antibody to German Cockroach by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BL1AN	235	6/29/2005	12/28/2006	0.340	0.000	0.0
BNMA4	165	6/29/2005	8/7/2006	7.582	0.665	8.8
BNMA4	165	6/29/2005	8/7/2006	12.685	1.278	10.1
BNMAB	82	8/10/2006	2/7/2007	8.428	0.631	7.5
BNMAB	82	8/10/2006	2/7/2007	20.811	1.943	9.3
BL1AY	12	1/4/2007	2/7/2007	0.340	0.000	0.0

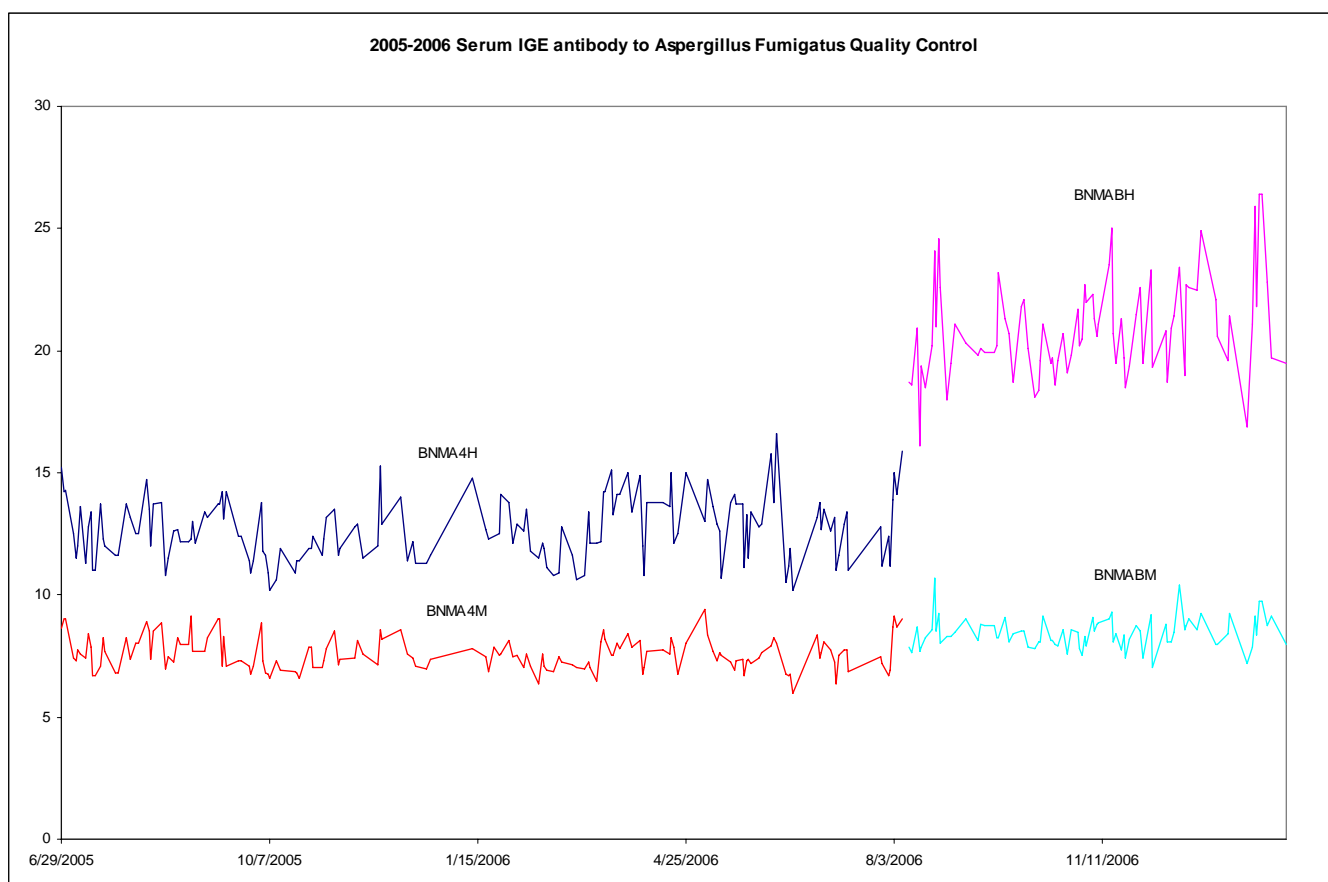


Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

M. Serum IGE antibody to Aspergillus Fumigatus

Summary Statistics for Serum IGE antibody to Aspergillus Fumigatus by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BL1AN	235	6/29/2005	12/28/2006	0.340	0.000	0.0
BNMA4	165	6/29/2005	8/7/2006	7.588	0.671	8.8
BNMA4	165	6/29/2005	8/7/2006	12.683	1.285	10.1
BNMAB	83	8/10/2006	2/7/2007	8.444	0.644	7.6
BNMAB	83	8/10/2006	2/7/2007	20.878	2.026	9.7
BL1AY	13	1/4/2007	2/7/2007	0.340	0.000	0.0

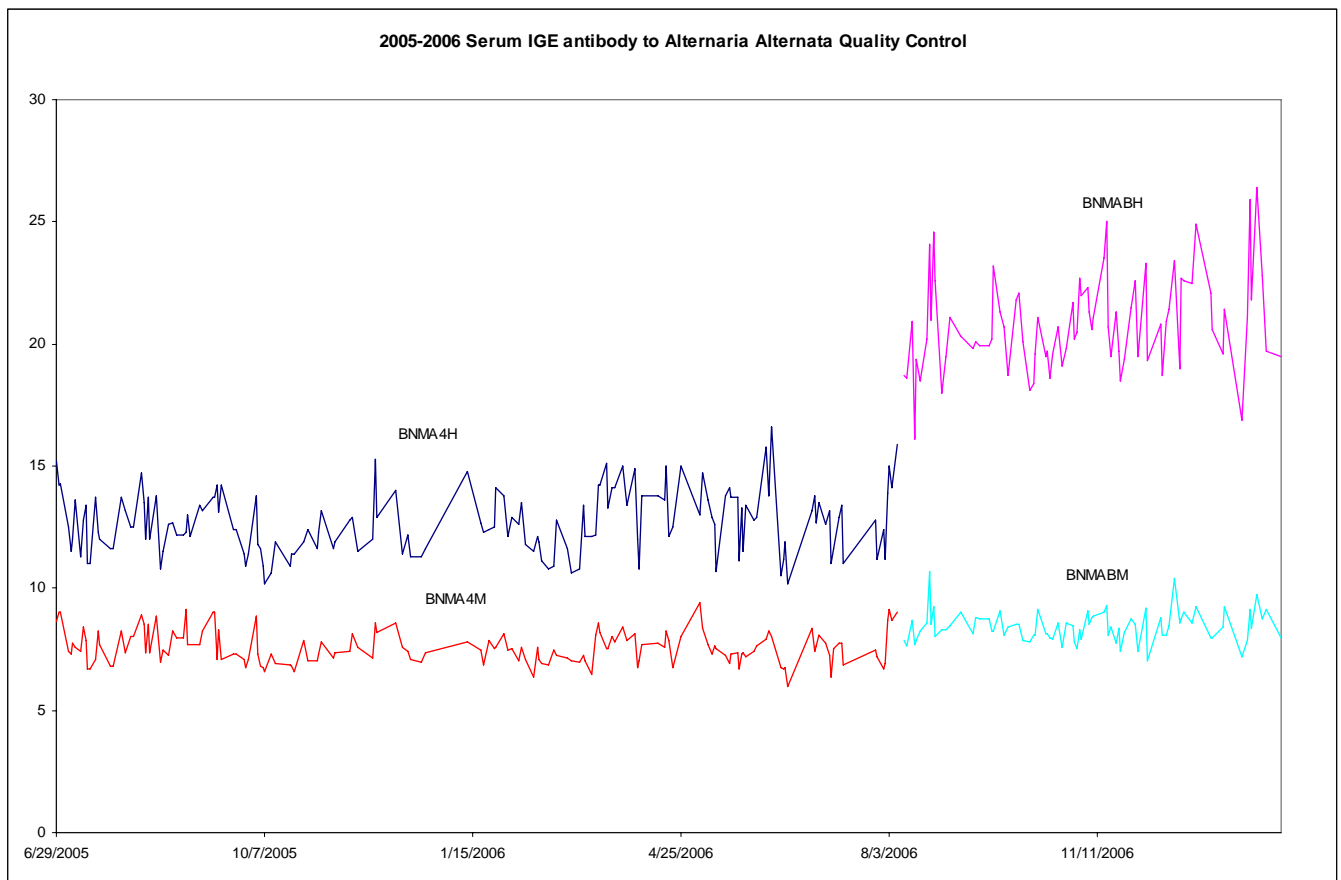


**Specific IgE/Total IgE Allergens in Serum
NHANES 2005-2006**

N. Serum IGE antibody to Alternaria Alternata

Summary Statistics for Serum IGE antibody to Alternaria Alternata by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BL1AN	233	6/29/2005	12/28/2006	0.340	0.000	0.0
BNMA4	163	6/29/2005	8/7/2006	7.577	0.670	8.8
BNMA4	163	6/29/2005	8/7/2006	12.682	1.291	10.2
BNMAB	82	8/10/2006	2/7/2007	8.428	0.631	7.5
BNMAB	82	8/10/2006	2/7/2007	20.811	1.943	9.3
BL1AY	12	1/4/2007	2/7/2007	0.340	0.000	0.0

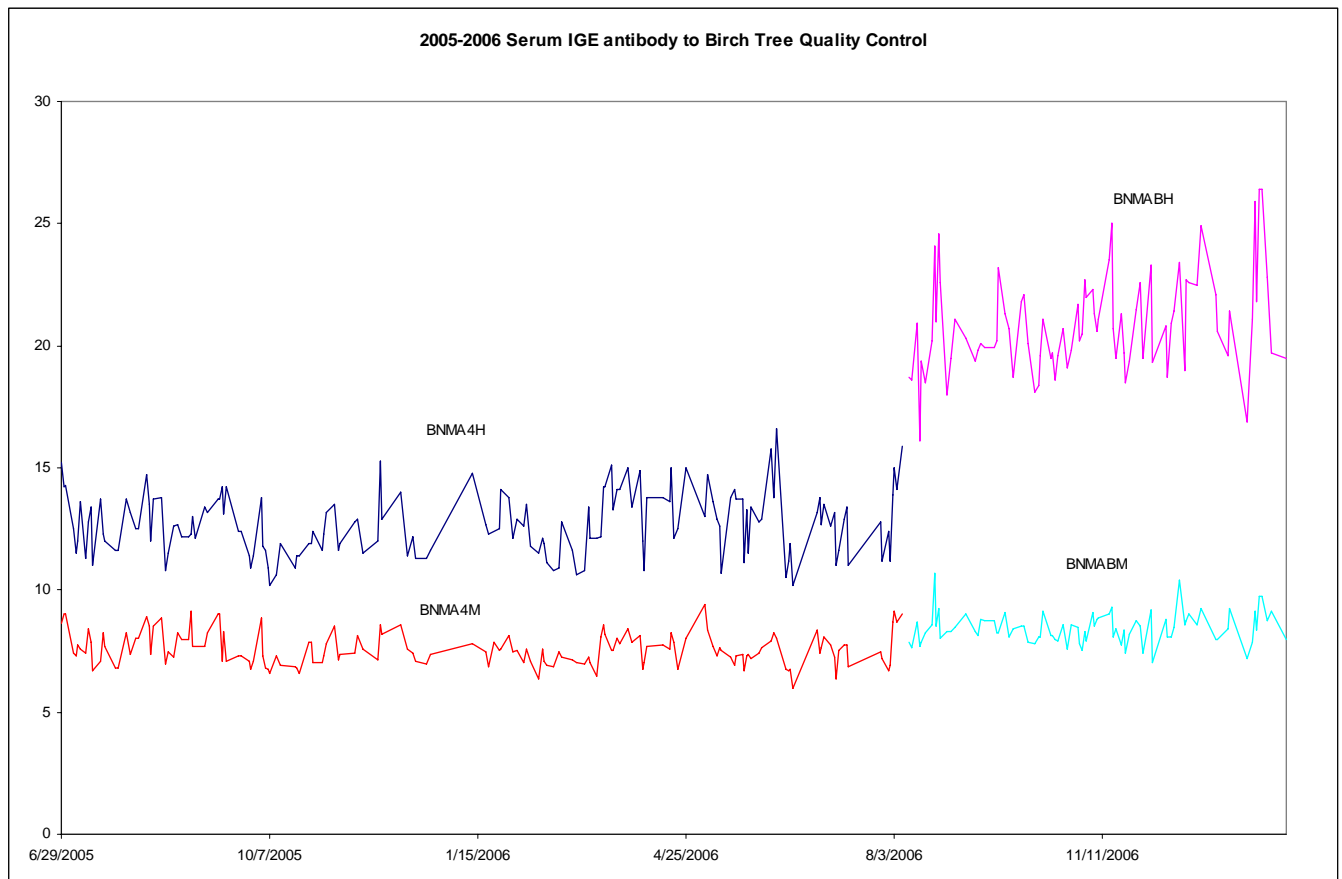


Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

O. Serum IGE antibody to Birch Tree

Summary Statistics for Serum IGE antibody to Birch Tree by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BL1AN	234	6/29/2005	12/28/2006	0.340	0.000	0.0
BNMA4	163	6/29/2005	8/7/2006	7.591	0.671	8.8
BNMA4	163	6/29/2005	8/7/2006	12.696	1.286	10.1
BNMAB	84	8/10/2006	2/7/2007	8.443	0.641	7.6
BNMAB	84	8/10/2006	2/7/2007	20.861	2.020	9.7
BL1AY	13	1/4/2007	2/7/2007	0.340	0.000	0.0

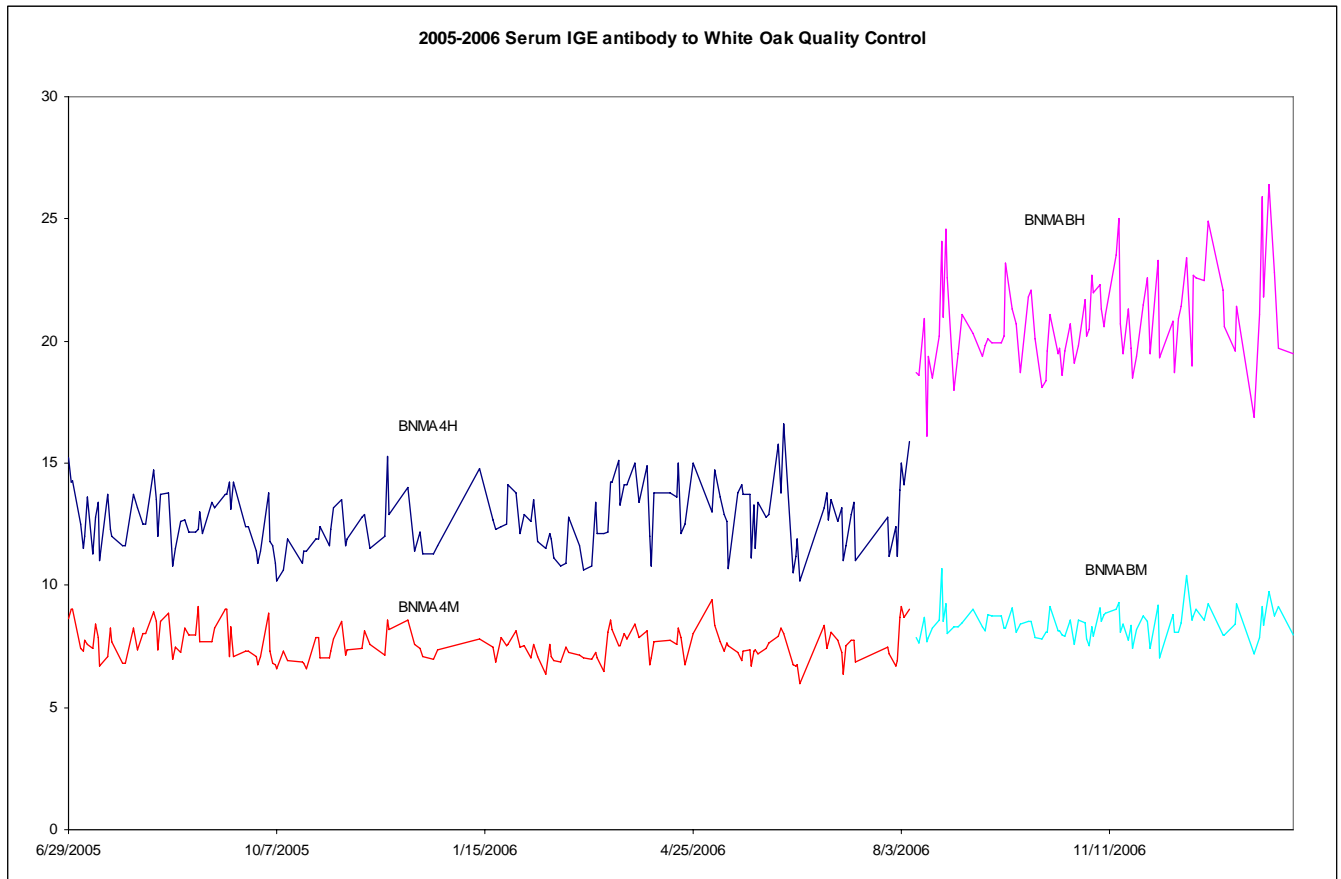


**Specific IgE/Total IgE Allergens in Serum
NHANES 2005-2006**

P. Serum IGE antibody to White Oak

Summary Statistics for Serum IGE antibody to White Oak by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BL1AN	234	6/29/2005	12/28/2006	0.340	0.000	0.0
BNMA4	163	6/29/2005	8/7/2006	7.591	0.671	8.8
BNMA4	163	6/29/2005	8/7/2006	12.696	1.286	10.1
BNMAB	83	8/10/2006	2/7/2007	8.427	0.628	7.4
BNMAB	83	8/10/2006	2/7/2007	20.794	1.937	9.3
BL1AY	12	1/4/2007	2/7/2007	0.340	0.000	0.0

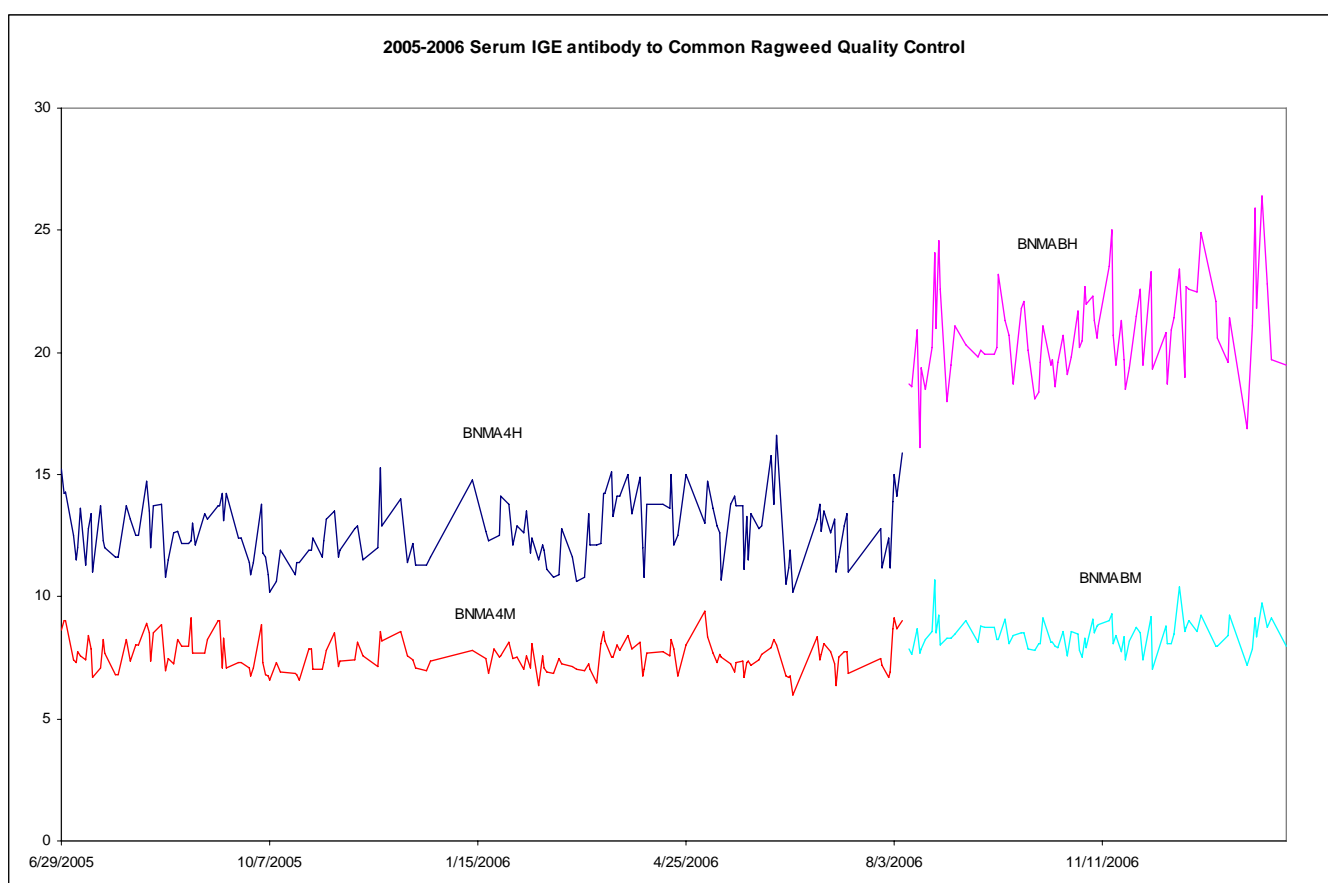


Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

Q. Serum IGE antibody to Common Ragweed

Summary Serum IGE antibody to Common Ragweed by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BL1AN	234	6/29/2005	12/28/2006	0.340	0.000	0.0
BNMA4	164	6/29/2005	8/7/2006	7.594	0.671	8.8
BNMA4	164	6/29/2005	8/7/2006	12.695	1.282	10.1
BNMAB	82	8/10/2006	2/7/2007	8.428	0.631	7.5
BNMAB	82	8/10/2006	2/7/2007	20.811	1.943	9.3
BL1AY	12	1/4/2007	2/7/2007	0.340	0.000	0.0

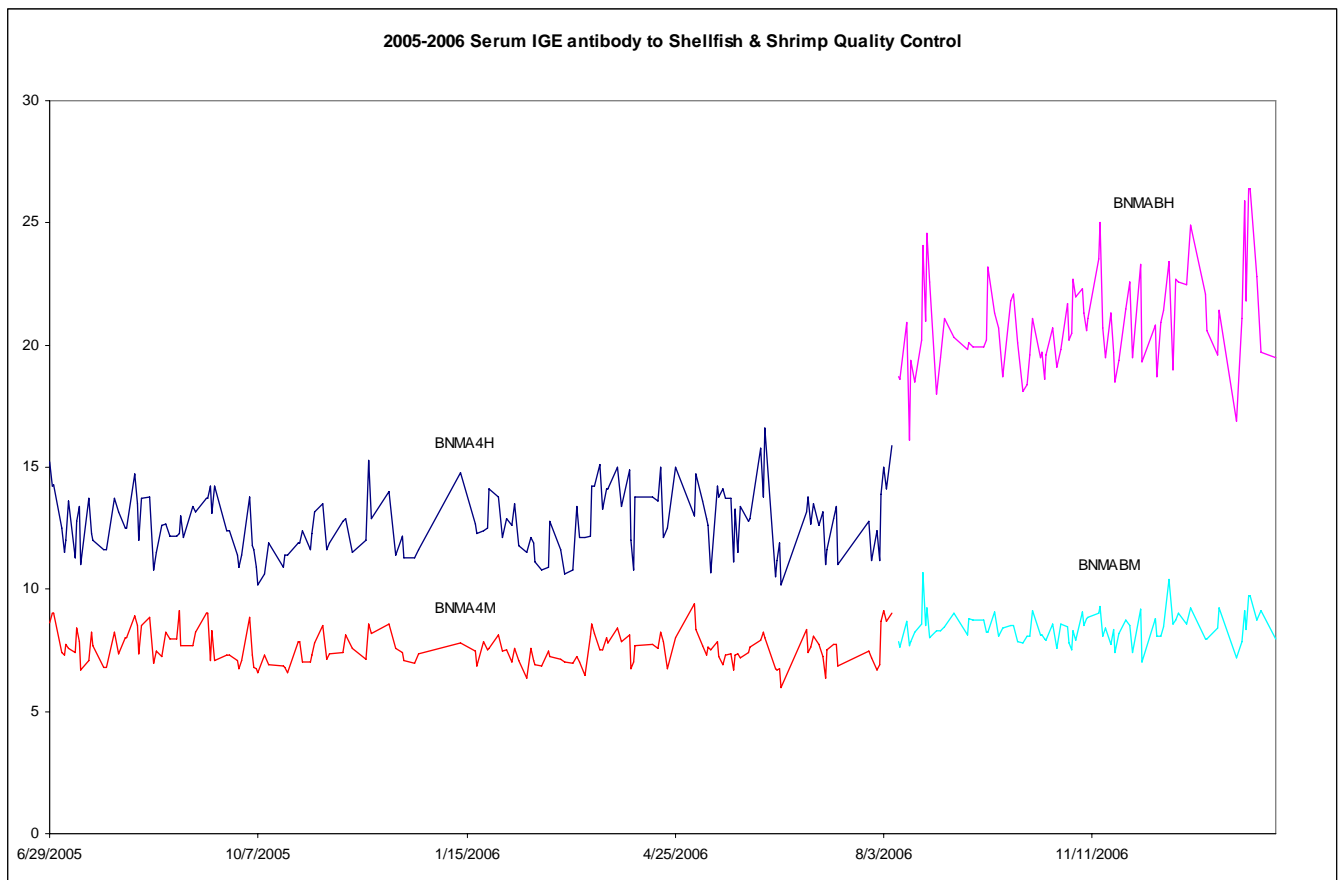


**Specific IgE/Total IgE Allergens in Serum
NHANES 2005-2006**

R. Serum IGE antibody to Shellfish & Shrimp

Summary Statistics for Serum IGE antibody to Shellfish & Shrimp by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BL1AN	234	6/29/2005	12/28/2006	0.340	0.000	0.0
BNMA4	164	6/29/2005	8/7/2006	7.593	0.670	8.8
BNMA4	164	6/29/2005	8/7/2006	12.705	1.287	10.1
BNMAB	83	8/10/2006	2/7/2007	8.444	0.644	7.6
BNMAB	83	8/10/2006	2/7/2007	20.878	2.026	9.7
BL1AY	13	1/4/2007	2/7/2007	0.340	0.000	0.0

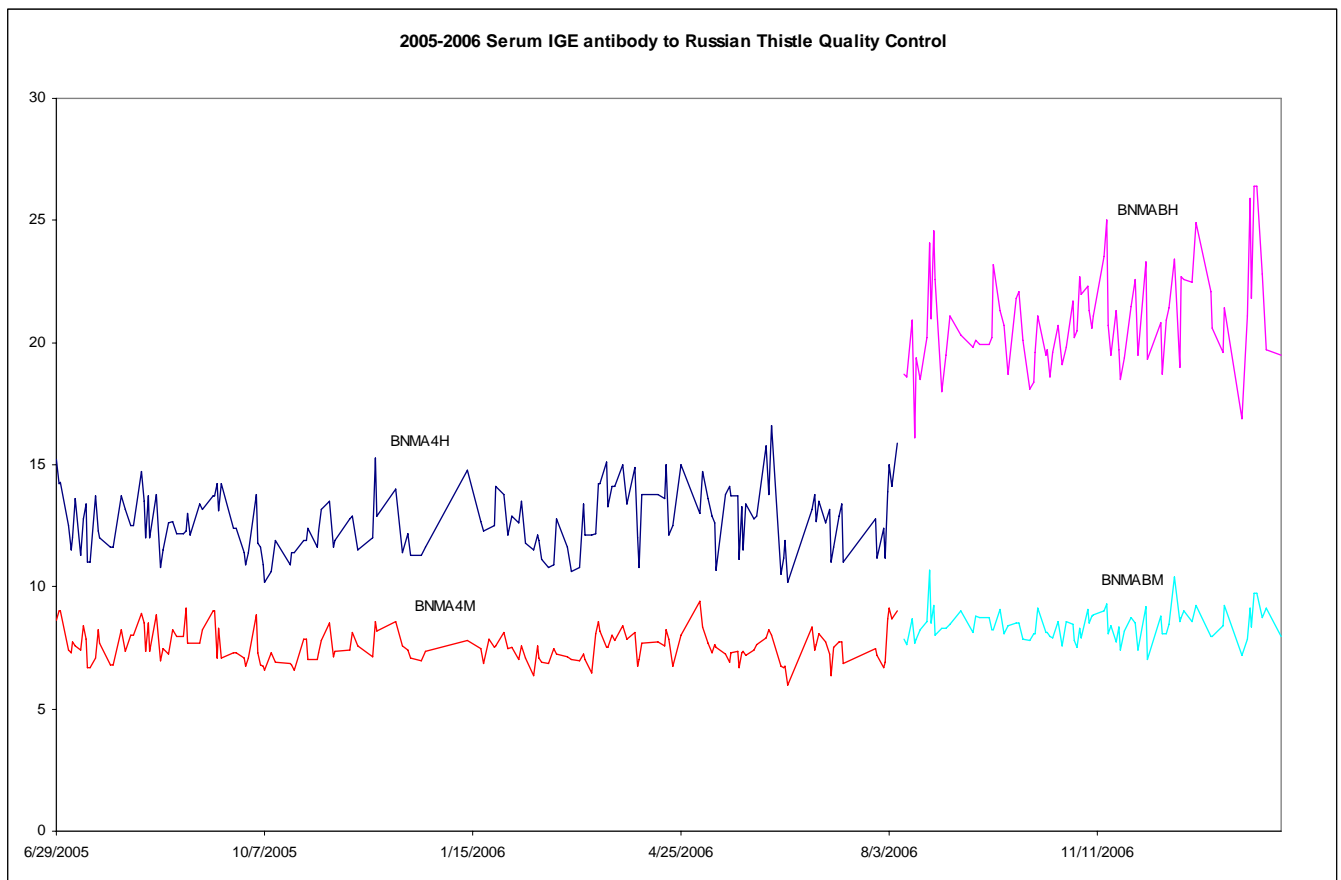


**Specific IgE/Total IgE Allergens in Serum
NHANES 2005-2006**

S. Serum IGE antibody to Russian Thistle

Summary Statistics for Serum IGE antibody to Russian Thistle by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BL1AN	235	6/29/2005	12/28/2006	0.340	0.000	0.0
BNMA4	165	6/29/2005	8/7/2006	7.584	0.671	8.8
BNMA4	165	6/29/2005	8/7/2006	12.682	1.286	10.1
BNMAB	83	8/10/2006	2/7/2007	8.444	0.644	7.6
BNMAB	83	8/10/2006	2/7/2007	20.878	2.026	9.7
BL1AY	13	1/4/2007	2/7/2007	0.340	0.000	0.0

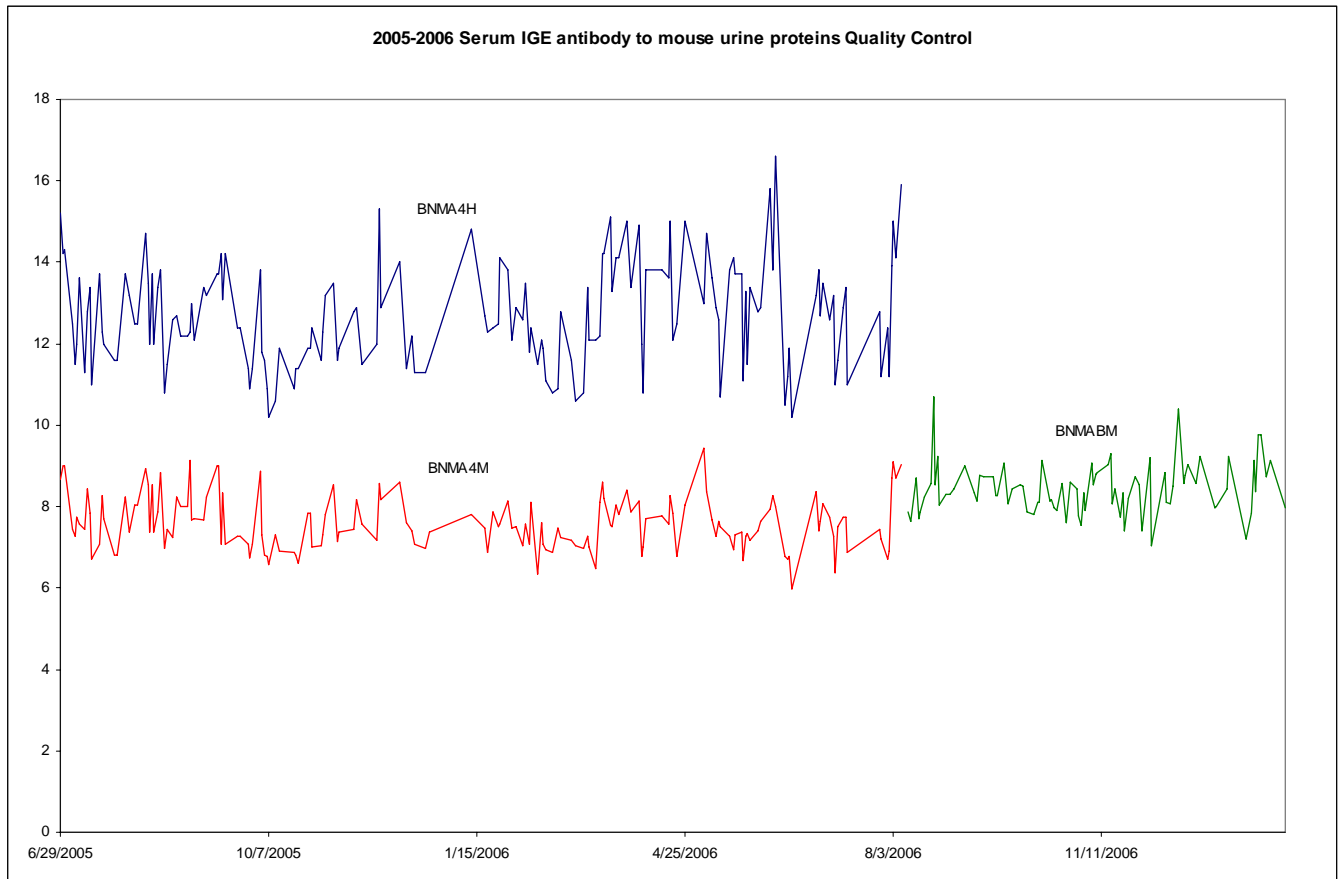


**Specific IgE/Total IgE Allergens in Serum
NHANES 2005-2006**

T. Serum IGE antibody to mouse urine proteins

Summary Statistics for Serum IGE antibody to mouse urine proteins by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
BL1AN	237	6/29/2005	12/28/2006	0.340	0.000	0.0
BNMA4	166	6/29/2005	8/7/2006	7.594	0.667	8.8
BNMA4	166	6/29/2005	8/7/2006	12.695	1.277	10.1
BNMAB	84	8/10/2006	2/7/2007	8.443	0.641	7.6
BNMAB	84	8/10/2006	2/7/2007	20.861	2.020	9.7
BL1AY	13	1/4/2007	2/7/2007	0.340	0.000	0.0



Specific IgE/Total IgE Allergens in Serum NHANES 2005-2006

References

1. Pharmacia Diagnostics package inserts.
1. Pharmacia Diagnostics System User Manual.
2. Bryant, Laboratory Immunology & Serology third edition, W.B. Saunders Company, 1992, pg. 33.
3. Turgeon, Immunology and serology in laboratory medicine, C.V. Mosby Company, 1990, pg. 259-260.
4. Bubak, M.E.; Reed, C.E.; Fransway, A.F.; Yunginger, J.W.; Jones, R.T.; Carlson, D.A. and Hunt L.W. : Subspecialty Clinics: Allergic Diseases – Allergic Reactions to Latex Among Health Care Workers. Mayo Clin Proc 67:1075-1079, 1992.
6. Slater, J.E. :Latex allergy. Allergy Clin Immunol94:139-149, 1994
7. Eriksson, N.E.: Diagnosis of IgE mediated allergy in clinical practice. Allergol .ET Immunopathol. 22,