Development and Use of a Method for Detection of IgE Antibodies to Cry9C

Richard B. Raybourne, Ph.D.
Immunobiology Branch
Office of Applied Research and Safety Assessment
Center for Food Safety and Applied Nutrition
Food and Drug Administration

June 13, 2001

Table of Contents

Executive Summary

Introduction

Type of Method

Optimizing the sensitivity and specificity of the ELISA for Cry9C

Controls for detection of bound human IgE

Testing of sera provided by CDC

Positive/negative system controls included within the ELISA

Replication of the ELISA

Limitations

Alternatives

Conclusions

Attachments

Attachment A: memorandum from K. Klontz to the record, dated 6/11/01

Attachment B: ELISA Protocol

Attachment C: Figure 1

Attachment D: Figure 2

Attachment E: Figure 3

Attachment F: memorandum from R. Raybourne to C. Rubin dated 5/23/01 and attachments

Attachment G: memorandum from R. Raybourne to C. Rubin dated 5/24/01 and attachments

Executive Summary

In early October of 2000, the Environmental Protection Agency (EPA) requested the assistance of the Food and Drug Administration (FDA) in assessing the significance of Adverse Event Reports (AERs) received by FDA and EPA in which consumers described adverse reactions they associated with consumption of corn products. In the AERs, many of the consumers linked their symptoms specifically to the purported presence of StarLink™ corn in the food they had consumed. FDA had received a cluster of such reports in September after articles in major newspapers announcing that traces of StarLink™ corn had been detected in some taco shells sold in grocery stores. StarLink™ corn is a variety of corn produced through recombinant DNA technology that contains a pesticidal protein known as Cry9C. On October 25, 2000, FDA requested the assistance of the Centers for Disease Control and Prevention (CDC) in evaluating these reported cases of human illness, which most of the consumers had characterized as an allergic reaction

FDA and CDC reported on the initial stages of their efforts at the November 28, 2000 meeting of EPA's Scientific Advisory Panel (SAP), and the conclusion that some of the AERs described symptoms and other details indicative of a possible allergic reaction. At the November 28 meeting, CDC also presented a proposal for further work that included administering questionnaires to individuals whose symptoms and signs were consistent with an allergic reaction, obtaining medical records, and collecting blood (serum) samples for possible future testing. Specifically, CDC proposed that the serum sample be evaluated for antibodies to the Cry9C protein using a still to be developed test. The SAP endorsed this proposal and CDC proceeded to develop the framework for analysis of the eventual test results while FDA proceeded to develop the serological test itself, an enzyme-linked immunosorbent assay (ELISA).

FDA developed the test using serum from animals and adapted it for use with human sera. Once adapted for use with human sera, FDA, at CDC's request, used the test to analyze sera provided by CDC. The serum samples provided by CDC consisted of sera from the individuals who had submitted AERs, historically banked serum samples collected before Cry9C entered the food supply, and also other serum samples from individuals known to be highly sensitive to a variety of allergens. To assure reproducibility, FDA had the testing replicated by an independent laboratory.

This report describes the factors taken into consideration in developing the ELISA. Strengths and limitations of the test are also described, including the lack of human serum from an individual known to be allergic to Cry9C to serve as a positive control. Data obtained from the testing at the FDA laboratory and at the independent laboratory are also presented.

Introduction

This report describes factors taken into account and specific procedures used in developing a method for detection of IgE antibodies to Cry9C protein. The need to develop a method capable of detecting IgE antibodies to the Cry9C protein was based on the reported occurrence of a number of adverse reactions in consumers who ingested food products that may have contained StarLink™ corn. Some of these reactions displayed symptoms consistent with allergic reactions caused by a type of antibody known as Immunoglobulin E (IgE). The vast majority of immediate allergic reactions to foods are due to IgE-mediated hypersensitivity (see Attachment A: memo to the record, K. Klontz, 6/4/01).

Type of Method

One of the most widely used methods for detecting the presence of IgE antibodies is an enzyme-linked immunosorbent assay (ELISA). In an ELISA, antigen is adsorbed or bound to the surface of specially treated plastic wells arranged in an 8 X 12 grid (ELISA plate). If antibodies that react with the antigen on the plate are present in serum, they will bind to the antigen. The presence of these bound antibodies is detected by the addition of a second antibody that reacts with the first antibody. It is at this level that specificity for IgE is introduced by using a second antibody that reacts only with IgE. The second antibody is labeled with an enzyme such as peroxidase or alkaline phosphatase. The presence of the bound second antibody is revealed by addition of the substrate for the enzyme along with an indicator that produces a color reaction in proportion to the amount of substrate degraded. The amount of color in each well, which is proportional to the amount of antibody, is measured as absorbance at a specific wavelength using an ELISA plate reader.

Optimizing the sensitivity and specificity of the ELISA for Cry9C

Several factors are important in optimizing this type of assay. The concept of optimizing the assay involves adjusting the components of the procedure to achieve the highest possible signal to noise ratio. One of the most critical elements is the amount of antigen used to coat the wells of the ELISA plate. Too much antigen will result in increased background (noise) and too little will result in loss of signal. Other important factors include the concentration of the second antibody and the agent(s) used to inhibit nonspecific binding of antibody. These agents include a protein used to block the unused antigen binding sites in the wells (blocking agent). Surface-active reagents such as Tween are also incorporated in the washing buffer along with protein to inhibit background.

It was recognized from the outset that a significant obstacle in the development of the ELISA was the lack of any known human sera with specific IgE for Cry9C. Nevertheless, the existence of a specific goat antiserum against Cry9C, and the availability of purified recombinant Cry9C made it possible to determine optimal conditions for coating of the ELISA plates and detection of bound goat antibody. Aventis

CropScience supplied recombinant Cry9C protein derived from *Escherichia coli*. Aventis also supplied a specific goat antiserum against Cry9C protein. Details regarding these and other materials used are contained in the Attachment B: ELISA Protocol.

The first step in the method development was to determine the optimal range of conditions, in terms of signal to noise, for detection of bound goat antibody. To accomplish this, a block, or grid, titration of Cry9C and goat antiserum was done. In this case, a block titration refers to testing all possible combinations across a range of protein concentrations (Cry9C) and a range of serum dilutions (immune goat serum). Serial dilutions of Cry9C protein were applied to Immunlon 1 and Immunlon 2 ELISA Plates. These plates differ in their protein binding characteristics based on proprietary chemistry of the manufacturer. When working with a new antigen, it is our normal procedure to try both Immulon 1 and Immulon 2 plates to determine which is best in terms of sensitivity and background. These experiments revealed that Immunlon 1 plates were optimal for low background.

Next, antigen was diluted in a pH 9.6 carbonate-bicarbonate buffer recommended by the manufacturer of the plates (see Attachment B: ELISA Protocol). After the antigen was removed, a solution of heat inactivated fetal bovine serum was added to the wells to occupy any remaining protein binding sites. Serial dilutions of normal and immune goat serum were applied to the various amounts of Cry9C in the wells. Some wells on each plate were left untreated by serum as sample diluent blanks. After incubation and washing, replicate plates were treated with 4 different dilutions of a second antibody directed against goat immunoglobulin. In order to test the efficacy of 2 different detection systems, antibody conjugated to either peroxidase or alkaline phophatase was tested. ELISA reactions were developed with the corresponding substrate and read on the ELISA plate reader. Use of peroxidase generally results in greater sensitivity, but sometimes is associated with higher background. Background readings were low in the Cry9C ELISA using peroxidase; thus the peroxidase substrate was used in all subsequent experiments.

Finally, the optimal concentration range of Cry9C to be used for coating ELISA plates was determined by maximizing signal to noise of immune versus normal goat serum. Antigen concentrations of as high as 100 micrograms/ml and as low as 0.049 micrograms/ml were tested. The optimal protein concentration range for Cry9C coating of the plates was between 3.125 and 0.78 micrograms/ml. This rather broad range indicates that the ELISA would tend to be very stable in terms the effects of variation in antigen concentration. In this range, ELISA readings for positive reactions with the immune goat serum were approximately one order of magnitude higher than the equivalent dilution of normal goat serum. Subsequently, the goat antiserum was titered and the endpoint dilution was determined to be greater than 1:1,000,000 indicating the ELISA is quite sensitive and specific for goat antibodies.

Controls for detection of bound human IgE

It was essential to demonstrate that the reagents used for detection of bound human IgE were functioning properly. In the absence of a known human IgE against Cry9C, the approach taken was to test for the ability to detect bound IgE directed against other human allergens such as cat and grass. To accomplish this it was necessary to establish optimal conditions for detecting IgE bound to these antigens. For this purpose, block titrations of cat and grass allergens and human sera from individuals known to be allergic to cat and grass was performed.

Serial dilutions of cat and grass allergens were coated on plates and incubated with serial dilutions of human sera from cat and grass sensitive individuals or from individuals with no known sensitivity to cat or grass. Human serum dilutions were kept low (no more than 1:8) to maximize the potential to detect low levels of IgE. After incubation and washing, replicate plates were incubated with 4 dilutions of goat anti-human IgE peroxidase conjugate. These experiments established an optimal concentration range of 25-100 micrograms/ml for coating ELISA plates with cat and grass allergens. These known allergic sera and their antigens were included as internal controls in subsequent tests, where they were used to demonstrate the ability of the goat anti-human IgE to detect bound human IgE (see Attachment B: ELISA Protocol).

At the same time as the cat and grass allergen controls were developed, it was determined that a food allergen should also be used to demonstrate that the reagents used for detection of bound human IgE were functioning properly. Cat and Grass antigens were available from FDA Center for Biologics, however, peanut antigen was not readily available. In the interest of providing timely results, cat and grass were incorporated into the protocol (see Attachment C: ELISA protocol). Subsequently the FDA laboratory produced peanut antigen (an aqueous extract of roasted peanuts). Similar procedures to those listed above were used to optimize conditions for coating of ELISA plates with peanut antigen. Positive control sera were obtained from peanut allergic individuals. Sera of known allergic individuals were obtained from IBT reference laboratories. This ELISA was run on 14 peanut positive sera using the same dilution, blocking, washing and second antibody reagents that were used in the Cry9C ELISA. However, the peanut ELISA was not included with the Cry9C ELISA as was done for cat and grass. A range of positive reactions was obtained with the sera from peanut allergic individuals, some of which were very strong (see Attachment D: Figure 2). Two points can be taken from these data on sera obtained from peanut allergic individuals: 1) the reagents and conditions used in the FDA laboratory can detect IgE antibody from food-allergic individuals; and 2) both strong and weak IgE responses were detected.

Testing of sera provided by CDC

When the ELISA procedure was optimized with respect to binding of Cry9C to the plates, sensitivity of detection of the goat antiserum, and detection of human IgE to

control cat and grass allergens, testing of samples received from CDC was started. Sera from CDC were received as coded samples with no personal identifiers. These sera included samples from individuals reporting adverse events allegedly linked to consumption of StarLink™ corn, sera from individuals allergic to other proteins (atopic sera) and samples of banked sera from EIS officers, collected prior to 1997. This date was chosen because it is prior to the introduction of StarLink™ corn to the market. All samples were tested in duplicate.

Several steps were taken to maximize the chances of detecting the presence of Cry9C-specific IgE. First, all sera were run in duplicate at a 1:2 dilution. Second, the amount of available antigen was increased to as high a level as possible, without increasing background in the ELISA. ELISA plates were coated with a 2 micrograms/ml solution of Cry9C, which is near the upper limit of the optimum range. This amount of Cry9C per ELISA plate well is more than 10 times the amount used for titration of the immune goat serum. The combined effect of these measures would be to increase the chances for a false positive, as opposed to a false negative result. Data obtained for the coded samples were provided to CDC for analysis (see Attachment F: memorandum from R. Raybourne to C. Rubin dated 5/23/01 and attachments). CDC decoded the samples, analyzed the data, and produced a figure showing the results of FDA's testing, identified by source (cases, pre-1996 controls, and atopic controls) (see Attachment C: Figure 1).

Positive/negative system controls included within the ELISA

In addition to the coded control sera provided by CDC, other control sera were also tested. These included sera with high (4132-11590 IU/ml) and low (6-34 IU/ml) total IgE. The high IgE sera would be types of serum most likely to cause a false positive reaction, and were included to test the susceptibility of the ELISA to this type of false positive. In addition, sera from patients allergic to a variety of allergens collected prior to 1997 were tested. To confirm the presence of Cry9C on the plates, standard dilutions (1:5000) of normal and immune goat sera were included on all plates as were blanks containing only sample diluent (no serum). To confirm reactivity of the anti-human IgE peroxidase conjugate in the presence of bound IgE, some wells coated with cat and grass allergens were included on the plates and tested with known allergic sera (see Attachment E: Figure 2)

Replication of the ELISA

To provide assurance of reproducibility, arrangements were made to replicate the ELISA in an independent laboratory at the University of Maryland (see Attachment G: memorandum from R. Raybourne to C. Rubin dated 5/24/01 and attachments). This laboratory was provided with the protocol used in the FDA laboratory. FDA also provided the coded samples from CDC and all other sera used by FDA, ELISA plates coated with Cry9C, and cat and grass antigens. Data from the University of Maryland testing were also provided to CDC (see Attachment E: Figure 3)

Limitations

The goal of this effort was to develop a method for detection of human IgE that binds to Cry9C in as timely a manner as possible, so that information could be supplied, through CDC, to the persons reporting adverse events, and to EPA for consideration by its scientific advisory panel. The approach of an IgE-specific ELISA was chosen over other alternatives as the most rapid, robust, and acceptable type of method.

Some limitations were apparent from the outset, such as the lack of any known human serum containing antibody to Cry9C. The result is that the possibility of a false negative cannot be entirely dismissed in spite of our efforts to reduce this possibility, described above. As stated above, every reasonable effort was made to weight the method toward obtaining positive reactions.

Another potential problem was related to the use of recombinant Cry9C expressed in (i.e., derived from) the bacterium *Escherichia coli*. Recombinant proteins from this source are not glycosylated (i.e., they do not have carbohydrate molecules attached). The same protein expressed in the corn plant may be glycosylated. In the case of some allergens, the molecular structures recognized by IgE antibodies (epitopes) involve these carbohydrate molecules. Thus, it is possible that epitopes present on Cry9C in corn may not be present in the *E. coli*-derived protein. However, isolation of Cry9C from corn was not practical in the quantities needed to set up the ELISA method. Consequently, this approach was not pursued.

Alternatives

In the absence of time constraints, possibilities exist that may overcome some of these limitations. Another test platform could be developed, such as the CAP-ELISA, which utilizes a sponge-like antigen matrix, and may be more sensitive than the plate ELISA. A classic RAST, or radioallergosorbant test, which uses paper disks or sepharose beads to immobilize antigen, could also be developed. This method requires the use of radioactive iodine, and both methods require large quantities of antigen. The advantage of using large amounts of antigen is the increased availability of more antigen binding sites. The problem related to glycosylation may be approachable through the use of IgE-specific western blots. In this procedure, corn extracts with or without Cry9C could be separated on an acrylamide gel, the separated proteins would then be transferred to a membrane, and then reacted with human serum. Recombinant Cry9C could be run in parallel as a control. As noted, such an approach would require a substantial amount of StarLink™ corn flour to produce sufficient antigen. In addition, this method is not as suitable as the ELISA for rapid testing of many samples.

Conclusions

The results from the coded CDC samples show no evidence of enhanced IgE ELISA reactivity of the adverse event sera versus the pre-1997 control or atopic sera (Figure 1). Under the same test conditions, a Cry9C antiserum produced in a goat was strongly

reactive for IgG against Cry9C, indicating that Cry9C protein was present and immunologically reactive on the ELISA plates (Attachment D: Figure 1). In addition, sera from individuals allergic to cat, grass or peanut were positive for IgE against the corresponding allergen when run in parallel (Attachment E: Figure 2). This indicates that the reagents used were sufficient to detect IgE antibodies bound to specific antigen, if such antibodies are present.

Figure Legends:

Figure 1: ELISA for IgE antibody against Cry9C run on CDC samples. Cry9C. Results of three runs. Absorbance scale expanded to encompass only CDC samples. Taken from CDC report to FDA dated 6/11/01.

Figure 2: Combined results of 3 runs of theELISA for IgE antibodies to Cry9C and IgE antibodies to cat, grass, and peanut antigens. Reactivity of goat antiserum to Cry9C is also shown. Absorbance scale encompasses range for positive controls (cat, grass, peanut and goat antibodies). Taken from CDC report to FDA dated 6/11/01.

Figure 3: Results of ELISA for IgE antibody to Cry9C run at University of Maryland on CDC samples. Reactivity of goat antiserum to Cry9C is also shown. Absorbance scale encompasses range for positive control (goat antibody). Taken from CDC report to FDA dated 6/11/01.

Attachment A

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date June 11, 2001

From Medical Officer, Epidemiology Team, Division of Market Studies (HFS-728), Office of Scientific Analysis and Support

Subject Method Development for Identification of IgE Antibody against Cry9C Protein in StarLink Corn - Decision not to Test for IgG Antibody

To Record

In mid-October of 2000, the Environmental Protection Agency (EPA) requested FDA's assistance in assessing the significance of adverse event reports possibly linked to StarLink™ corn. FDA, in turn, requested assistance from the Centers for Disease Control and Prevention (CDC) in a collaborative effort to study the possible clinical significance of those reports. The initial results of the effort were presented at the November 28, 2000 meeting of EPA's Scientific Advisory Panel. Also presented by CDC was a proposal for further investigation. The proposal included establishment of a case definition; interviews with, and collection of medical records from, those consumers who met the case definition; and also collection of blood samples from consenting individuals. Blood samples were to be banked, pending development of a serological test to examine them for antibodies to Cry9C protein. (Such a test had not yet been developed at the time of the SAP meeting.) CDC and FDA received strong support from the SAP to pursue this avenue in assisting EPA, in its efforts to resolve questions regarding the potential allergenicity of Cry9C protein. After considering various options, FDA decided to develop the test as an enzyme-linked immunosorbent assay (ELISA) for IgE antibodies to Cry9C protein. This memorandum describes factors considered in deciding to focus on IgE antibodies only.

In the initial stages of designing a method to analyze human serum for antibodies that react with (i.e., bind to) Cry9C protein, FDA gave consideration to analyzing serum samples for both IgE and IgG antibodies. However, re-examination of both the scientific literature (see, for example, Binslev-Jensen and Poulsen, 1997; Sampson 1997; Sampson and Burks, 1996) and comments from members of the FIFRA Scientific Advisory Panel (SAP) from the meeting held November 28, 2000, suggested that analyzing for only IgE was the judicious approach to follow. For example, Sampson, 1997 noted: "The vast majority of immediate allergic reactions to foods are due to IgE-mediated hypersensitivity." This judgement is reflective of the views of most experts in this area. Further, the report of the November 28, 2000 meeting of the SAP specifically recommended: "serum should be obtained from these patients [those reporting adverse reactions] and evaluated for the presence of IgE antibody to Cry9C."

Consequently, during the early stages of protocol development, FDA focused greater attention on the need to analyze serum specimens for IgE against Cry9C than for IgG. With EPA agreement,

FDA and CDC held further discussions with selected SAP members who have expertise in immunology and food allergenicity, to seek their advice and determine whether this focus was advisable. The results of these discussions are summarized below.

In a February 20, 2001 meeting at the National Institutes of Health (NIH) with Dean Metcalfe, M.D. (NIH) met with Carol Rubin, DVM (CDC), Robert Vogt, Ph.D. (CDC), Richard Raybourne, Ph.D. (FDA), Kristina Williams, Ph.D., (FDA), and Karl Klontz, M.D. (FDA). FDA/CDC described to Dr. Metcalfe the proposed protocol for testing for antibodies to Cry9C protein, which at that time included the possibility of testing for IgG, and asked his advice regarding inclusion of IgG. Dr. Metcalfe suggested that the method be restricted to analyzing for only IgE antibody and provided the following rationale for this opinion: 1) it is IgE antibody that plays the critical clinical role in the genesis of true allergic reactions from foods, not IgG; and 2) there is a real possibility that cross-reacting IgG antibodies (IgG antibodies that react to more than one protein) could cloud the picture and add confusion as to the meaning of the IgG.

In late February and early March 2001, FDA and CDC independently reviewed scientific literature, took into account the preponderance of comments at the SAP meeting, and also considered the specific advice from Dr. Metcalfe. Ultimately, CDC decided to request that sera be tested only for IgE reactivity with Cry9C protein because IgE is the only type of antibody that causes immediate hypersensitivity in humans, and that any other antibody reactivity (IgG or IgA) would be irrelevant to the immediate-type allergic reactions specified in CDC's case definition.[a] Put in another way, any results of evaluations for IgG reactivity with Cry9C protein would not be clinically relevant to the case reports. (Affirmed in personal communications, R. Vogt and C. Rubin, CDC, and R. Raybourne, (FDA), March 13, 2001.) Consequently, the FDA focused its efforts exclusively on development of a method to detect IgE antibodies.

Personal communications between Marc Rothenberg M.D. Ph.D. (Allergy and Clinical Immunology, University of Cincinnati) and Richard Raybourne, Ph.D. provided additional support for the approach taken by CDC and FDA. Specifically, Dr. Rothenberg commented that the significance of IgG anti-dietary antigens is uncertain and that most studies have shown that this is a normal response. Further, that if Cry9C protein were shown to be immunoreactive (e.g., induces IgG production) but not allergenic (e.g., no IgE induction) that this may create a problem with interpretation and communication of test results. Consistent with this view, the results of a double-blind, placebo-controlled study (Morgan et al. 1990) of persons with shrimp-specific IgG subclass antibodies showed that while some shrimp-specific IgG subclass levels were increased in shrimp-sensitive subjects, none of the subclass responses were significantly predictive of a positive response to double blind placebo-controlled challenge and therefore were

---

[a] CDC's case definition: An adverse health event which occurred 1 July through 30 November 2000, temporally linking corn product consumption to anaphylactic symptoms within 1 hour of eating, dermatologic symptoms within 12 hours of eating, or gastrointestinal symptoms within 12 hours of eating. Excluded were those cases with gastrointestinal symptoms if more than one member of the group experienced similar symptoms after a common meal, considering these more likely due to an infectious or toxic foodborne etiology than to a food allergic reaction. (Winterton et al., 2001, EIS meeting presentation slides).

not diagnostic of shrimp intolerance.

Based on all of the information and advice described above, FDA focused its method development efforts on detecting anti-Cry9C IgE. Ultimately, CDC requested that FDA test the case samples and controls provided by CDC for IgE antibodies, only.

References

Binslev-Jensen, C. and L.K. Poulsen. 1997. *In vitro* diagnostic methods in the evaluation of food hypersensitivity, Chapter 7, pp. 137-150. In: Food Allergy: Adverse Reactions to Foods and Food additives, Second edition. Metcalfe, D.D., H.A. Sampson, and R.A., Simon (editors). Blackwell Science, Cambridge, MA.

Morgan, J.E., Daul C.B, Lehrer, S.B. 1990. The relationships among shrimp-specific IgG subclass antibodies and immediate adverse reactions to shrimp challenge. *Journal of Allergy and Clinical Immunology*. 86:387-392.

Sampson, H.A. 1997. Immediate reactions to foods in infants and children. Chapter 9, pp.169-182. In: Food Allergy: Adverse Reactions to Foods and Food additives, Second edition. Metcalfe, D.D., H.A. Sampson, and R.A., Simon (editors). Blackwell Science, Cambridge, MA.

Sampson, H.A., and A. W. Burks. 1996. Mechanisms of food allergy. Annu. Rev. Nutr. 15:161-177.

Karl C. Klontz, MD

Karl C. Klontz, M.D.
Medical Officer

Attachment B

PROCEDURE FOR ELISA DETECTION OF IgE ANTIBODIES TO CRY9C

COAT ELISA PLATES

1) Suspend purified Cry9C solution (supplied by Aventis Crop Science) to a concentration of 2 ug/ml in carbonate/bicarbonate buffer, pH 9.6. Suspend crude grass antigen to a concentration of 40 ug/well, and crude cat antigen to a concentration of 0.2 units/ml. (optimum concentration of antigen previous determined by block titration with known positive and negative goat antiserum, supplied by Aventis Crop Science)

2) Pipet 100 ul/well into Dynatech Immulon I plates. Include grass and cat antigen to serve as reagent controls.

3) Incubate overnight at 4C.

ADD SERA TO PLATES

4) Allow plates to equilibrate to RT.

5) Aspirate liquid from wells with 12-channel manifold. Wash plates 1X with phosphate buffered saline (PBS).

6) Block for 2 hrs. RT with PBS-10% heat inactivated fetal bovine serum (HIFBS), 100 ul/well.

7) Aspirate liquid from wells as above. Wash plates 2x with PBS.

8) Dilute sera 1:2 with sample diluent (PBS-5% HIFBS, 0.05% Tween 20). Dilute goat antisera 1:5000 with sample diluent.

9) Add diluted sera to wells, 100 ul/well, in duplicate. Pipet known positive sera from cat and grass allergic individuals, diluted 1:2, into cat and grass antigen-coated wells. Sera provided by IBT reference labs.

10) Incubate plates at RT for 2 hrs. or overnight at 4C.

ADD CONJUGATE

11) Allow plates to equilibrate to RT (if incubated at 4C).

12) Aspirate liquid from wells. Wash plates 4x with PBS-0.1% Tween-20 (wash buffer). Allow wash buffer to remain in wells for 1 min.

13) Add affinity purified peroxidase-conjugated goat anti-human IgE (KPL, Cat. #074-1002) to wells, 100 ul/well. For wells with goat serum, add affinity purified peroxidase-conjugated donkey anti-goat IgG (Jackson Labs, Cat. # 705-035-147). Appropriate dilution of conjugate must be determined for each new lot. Typical dilution is 1:2000 Conjugate is diluted in PBS-10% HIFBS.

14) Incubate 2 hrs. at RT.

DEVELOP COLOR

15) Aspirate liquid from wells and wash 4x as above with PBS-0.1% Tween 20.

16) Add 100 ul/well of substrate solution (TMB-Elisa, Gibco Labs, Cat. #15980-014).

17) Incubate 15 min. at RT.

18) Stop reaction with 100 ul/well 1N H_2SO_4.

19) Read absorbance with 96-well Elisa plate reader, 450 nm.

Attachment C

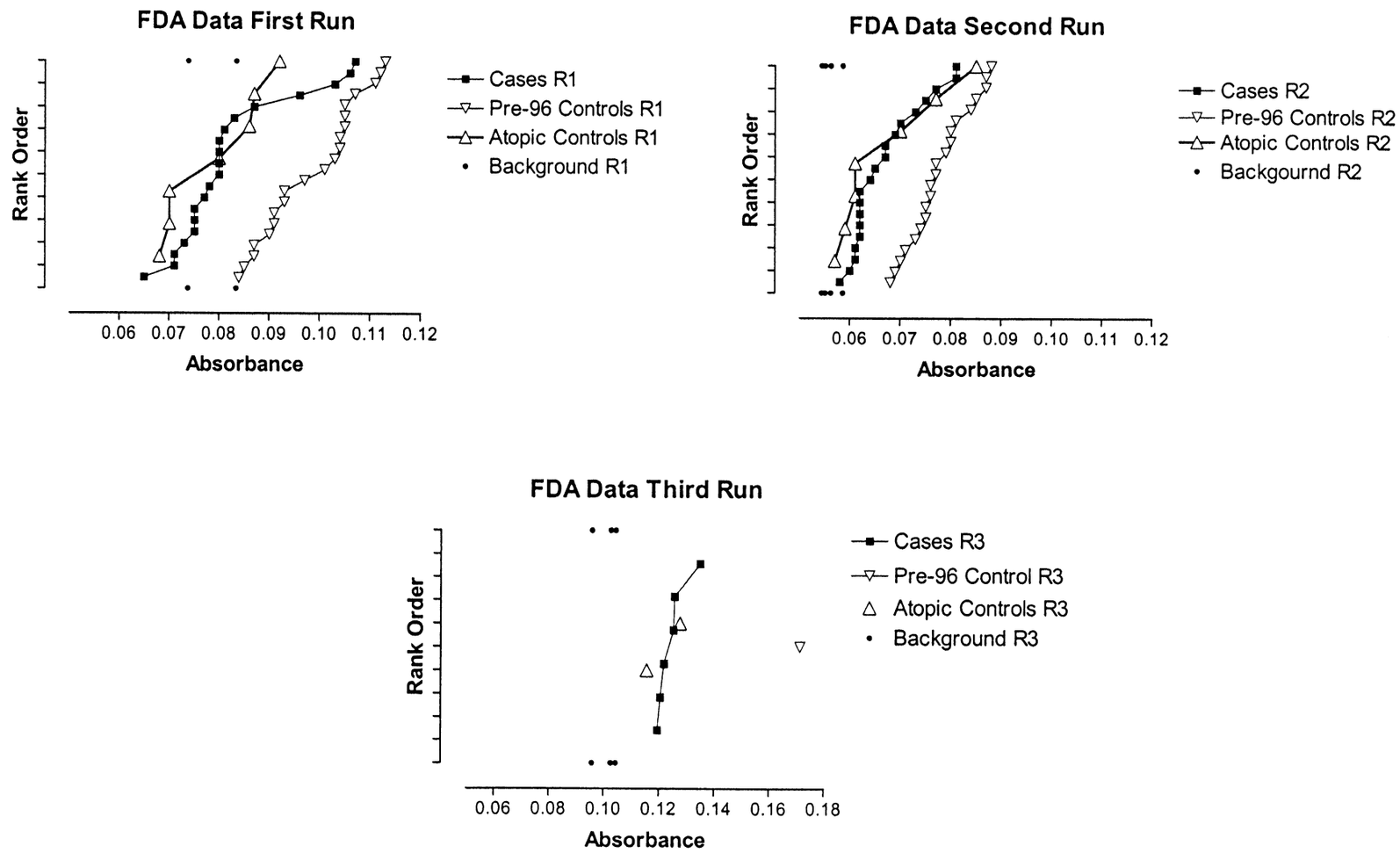
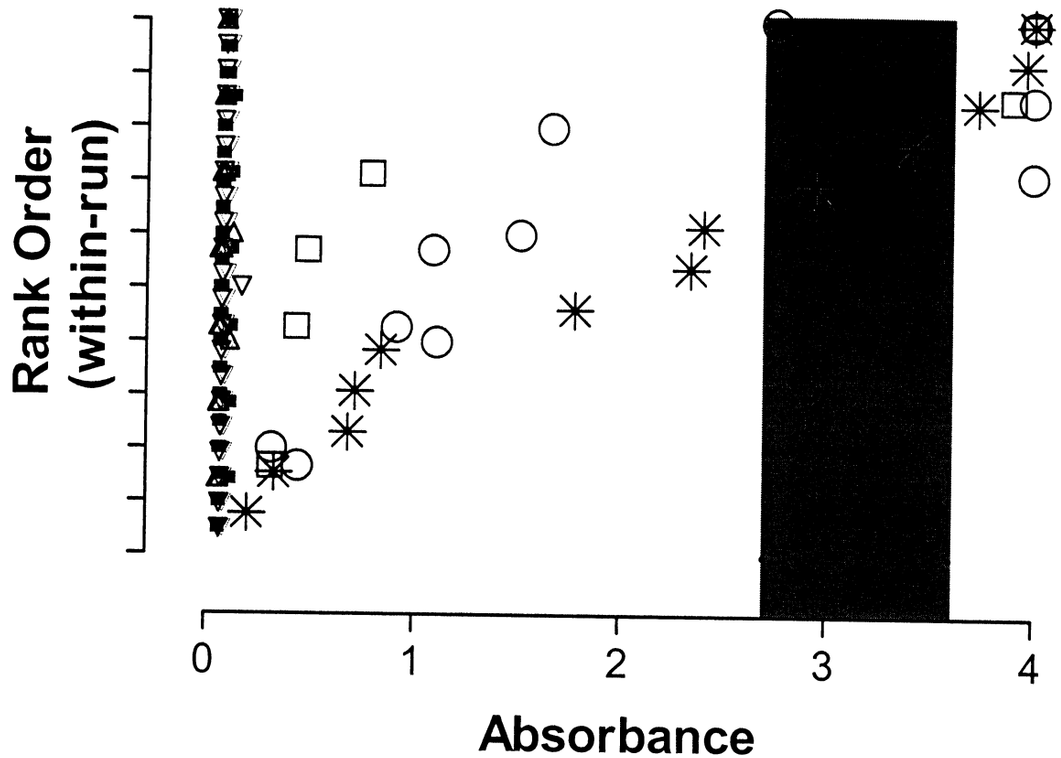


Figure 1

Attachment D



Cry9c – IgE Reactions

- ▪ Cases
- ▽ Pre-96 Controls
- △ Atopic Controls

Allergen – IgE Reactions

- ○ Grass Pos Controls
- □ Cat Pos Controls
- ✳ Peanut Pos Controls

■ **Hyperimmune anti-Cry9c Goat Serum**
(Overall Range for All Runs)

Figure 2

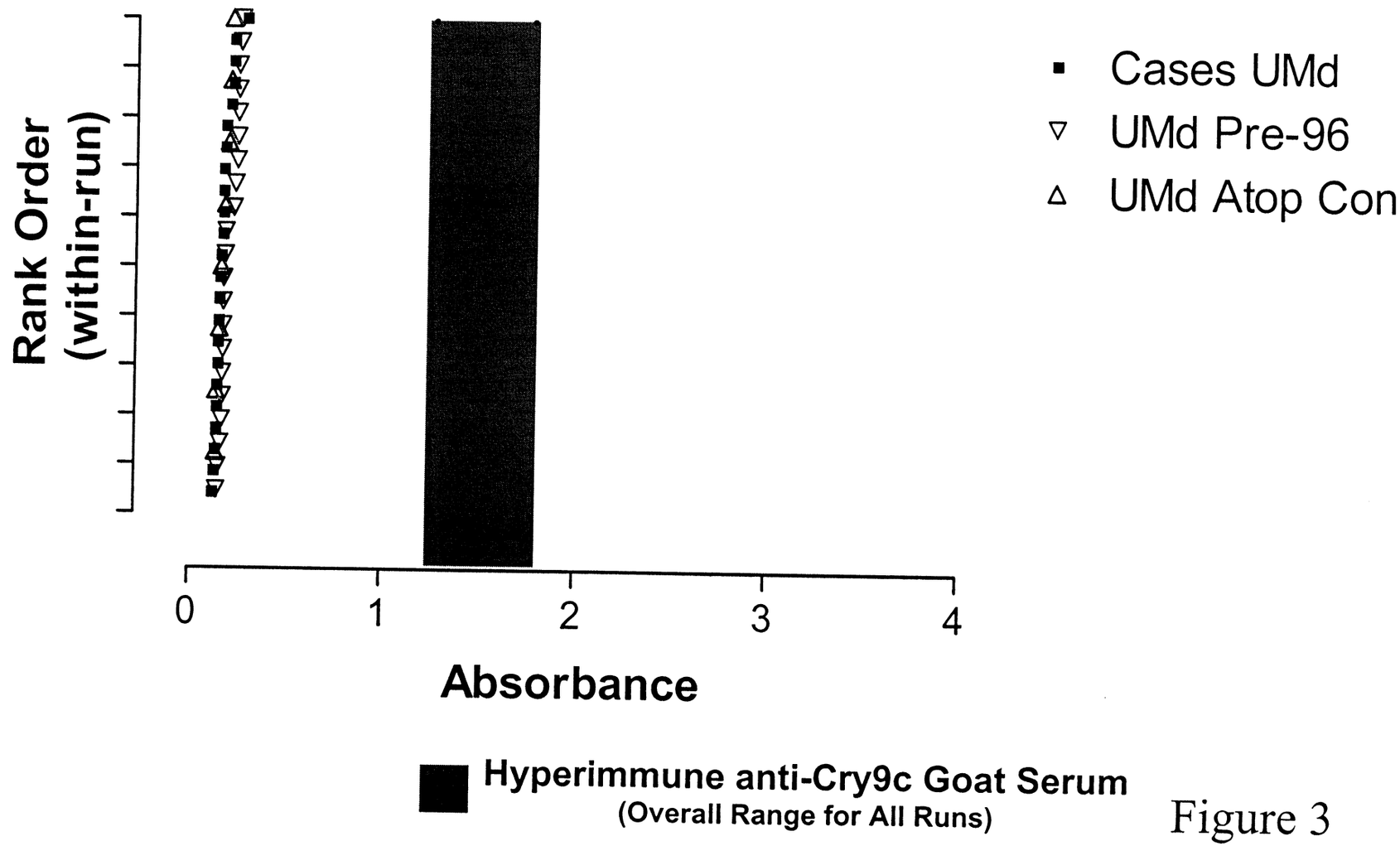

Rank Order
(within-run)
0
1
2
3
4
Absorbance
Cases UMd
UMd Pre-96
UMd Atop Con
Hyperimmune anti-Cry9c Goat Serum
(Overall Range for All Runs)

Figure 3

Attachment F

Date: 5/23/01

From: **Richard B. Raybourne, Ph.D.**
IB, DVA, OARSA, CFSAN, FDA

To: **Dr. Carol Rubin, CDC**

Subject: **ELISA results for human serum samples sent by CDC**

This is in response to your earlier request to analyze the coded samples provided to FDA using our experimental method designed to detect IgE antibody that binds to Cry9c.

Attached is a brief description of the method, which we consider to be still under development. Please note that no human serum known to contain IgE against Cry9c was available for use as a positive control. Included in the test protocol (attached) was a control antiserum against Cry9c raised in goats that assured that Cry9c protein was bound to the ELISA plate wells. To confirm reactivity of the anti-human IgE peroxidase conjugate in the presence of bound IgE, some wells coated with reference standards of cat and grass allergens were included on the plates and tested with known allergic sera to those antigens. In order to maximize the possibility of detecting Cry9c-specific IgE, all samples from CDC were run in duplicate at a 1:2 dilution.

The data provided on the enclosed disk was produced in the FDA laboratory and consists of duplicate optical density readings from the ELISA plate reader for all of the coded samples. A hardcopy of the electronic file is also provided for convenience. Also included are readings from control wells to which sample diluent without serum was added (diluent blank). These readings should be useful in allowing you to determine the inherent background in the system, and to provide a baseline against which to gauge the absolute reactivity of the coded samples. We can also supply the readings for the control sera mentioned above, if you wish.

To provide assurance of reproducibility, we have had the testing confirmed by an independent laboratory at the University of Maryland. We would be very happy to provide you with these data as well.

Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1
0.0858	0.0885	9	0.088	0.096	17	0.0876
0.0828	0.0862	10	0.0909	0.0914	18	0.0855
0.0694	0.0723	11	0.1039	0.0987	19	0.1052
0.066	0.0641	12	0.0675	0.0716	20	0.0864
0.102	0.106	13	0.1016	0.1223	21	0.088
0.075	0.0745	14	0.0948	0.0921	22	0.1066
0.0718	0.0776	15	0.0734	0.0731	23	0.1015
0.1099	0.102	16	0.0758	0.0746	24	0.078

Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1
0.0912	0.1008	41	0.0684	0.0675	49	0.0788
0.0849	0.0867	42	0.0909	0.0824	50	0.079
0.0891	0.0853	43	0.1048	0.1046	297	0.1136
0.068	0.0734	44	0.0804	0.0822	394	0.0816
0.095	0.1188	45	0.0752	0.0815	532	0.1128
0.1042	0.109	46	0.0852	0.0801	534	0.1146
0.0988	0.1108	47	0.1143	0.1079	804	0.135
0.0782	0.0767	48	0.0826	0.078	843	0.143

Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2
0.0979	25	0.0699	0.0724	neg. pool	0.0827	0.0817
0.0892	26	0.1014	0.1049	neg nordlee	0.0665	0.0704
0.1043	27	0.089	0.0926	DD	0.1179	0.1102
0.0817	28	0.0695	0.0697	TK	0.0871	0.0792
0.0923	29	0.0818	0.0782	DV	0.0896	0.095
0.1193	30	0.1039	0.102	o1 1	0.0881	0.1028
0.107	31	0.0933	0.1009	TB	0.1306	0.126
0.0826	32	0.112	0.0969	sample diluent	0.0834	0.0834

Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2
0.082	sample diluent	0.0784	0.0691
0.0811			
0.1056			
0.0874			
0.1093			
0.1015			
0.1248			
0.1398			

2

##BLOCKS= 2

Plate:	Plate#1			Cry9c	Cry9c	
	Temperature(°C)	sample		OD 1	OD 2	sample
	24.8	normal goat		0.0863	0.1029	1
		immune goat		3.7947	3.6486	2
		elms		0.0928	0.0993	3
			131	0.113	0.1009	4
			132	0.0844	0.0774	5
			281	0.0821	0.0911	6
			533	0.0754	0.0807	7
			993	0.0804	0.0829	8

~End

Plate:	Plate#2			Cry9c	Cry9c	
	Temperature(°C)	sample		OD 1	OD 2	sample
	24.4	normal goat		0.0802	0.0813	33
		immune goat		3.6932	3.5897	34
		control 9		0.0739	0.0766	35
			255	0.0666	0.0716	36
			315	0.0914	0.0939	37
			321	0.0897	0.0847	38
			661	0.0899	0.0936	39
			991	0.1257	0.1344	40

~End

##BLOCKS= 3

Plate: Plate#1

Temperature(°C)	sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1
24.5	normal goat	0.0706	0.0648	1	0.0675
	immune goat	2.7486	2.8164	2	0.0761
	9	0.0635	0.0608	3	0.062
	255	0.0627	0.059	4	0.0604
	315	0.0722	0.0692	5	0.0792
	321	0.0666	0.0642	6	0.0617
	661	0.0747	0.0729	7	0.0606
	991	0.0821	0.0817	8	0.0823

~End

Plate: Plate#2

Temperature(°C)	sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1
24.5	normal goat	0.0729	0.0724	32	0.0764
	immune goat	2.8934	2.9091	33	0.0795
	297	0.0885	0.087	34	0.0767
	394	0.0667	0.0754	35	0.0841
	532	0.0935	0.0876	36	0.0637
	534	0.0787	0.0716	37	0.0684
	804	0.1201	0.1079	38	0.0843
	843	0.1034	0.0963	39	0.0862

~End

Plate: Plate#3

Temperature(°C)	sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1
24.6	normal goat	0.0828	0.0766	m3 34965	0.0857
	immune goat	3.4272	3.4782	i1 28054	0.0864
	w10 18012	0.0812	0.0813	m5 29661	0.089
	m5 29654	0.1127	0.0945	e4 13891/2	0.0962
	e3 15521	0.0926	0.0864	f13 33295	0.0893
	f23 13379	0.1119	0.094	f1 19245	0.0949
	d1 35018	0.0881	0.083	f23 26224	0.0885
	e2 29065	0.0775	0.0812	d2 28555	0.0833

~End

4

Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2
0.0674	9	0.0752	0.0944	17	0.0726	0.0759
0.073	10	0.079	0.0728	18	0.0734	0.0675
0.0636	11	0.095	0.0784	19	0.0789	0.0745
0.0613	12	0.0599	0.059	20	0.0678	0.0672
0.0803	13	0.0812	0.0783	21	0.0759	0.0697
0.0619	14	0.0686	0.0688	22	0.075	0.0765
0.0618	15	0.0597	0.0571	23	0.0777	0.0732
0.0806	16	0.0625	0.0609	24	0.0654	0.0632

Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2
0.0776	40	0.0626	0.0613	48	0.0593	0.0625
0.0743	41	0.0612	0.0616	49	0.0703	0.0791
0.0769	42	0.0745	0.0759	50	0.0699	0.0758
0.0769	43	0.0853	0.0893	h2 27609	0.0806	0.0803
0.0668	44	0.0715	0.0689	g6 26589	0.0728	0.0695
0.0692	45	0.0619	0.0628	f2 29663	0.0843	0.084
0.0835	46	0.0624	0.0612	e1 26504	0.0955	0.0957
0.0835	47	0.0858	0.0904	t7 28312	0.0829	0.0765

Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2
0.089	i3 34965	0.0854	0.0788	m6 29396	0.0868	0.0887
0.0913	e2 161841/3	0.0969	0.1085	m1 33333	0.093	0.0845
0.0898	w10 28445	0.1009	0.0986	e4 18922	0.0742	0.071
0.1098	e3 27814	0.0852	0.0839	e4 16530	0.0916	0.0885
0.0969	w10 35390	0.0791	0.0769	f1 29600	0.0787	0.0768
0.0941	h2 26737	0.0839	0.0903	d2 35019	0.0912	0.0872
0.0894	d1 7569	0.0923	0.0851	e3 14338	0.0719	0.0708
0.0844	e1 27623	0.0977	0.1	i1 6763	0.0987	0.0965

sample	Cry9c OD 1	Cry9c OD 2	sample	grass OD 1	grass OD 2
25	0.0604	0.0591	131	2.7211	2.7853
26	0.0819	0.077	132	1.604	1.7301
27	0.0697	0.07	g6 27613	0.3029	0.3319
28	0.0546	0.0586	g8 14330	1.0812	1.1405
29	0.0672	0.0661	g6 24590	1.5446	1.4853
30	0.0838	0.0786	f13 35234	0.1753	0.1618
31	0.0702	0.0721	d2 25570	1.5198	1.3396
sample diluent	0.0554	0.0533	sample diluent	0.054	0.0561

sample	Cry9c OD 1	Cry9c OD 2	sample	cat OD 1	cat OD 2
m5 29633	0.0808	0.0792	281	1.7544	1.6966
c1 26218	0.0735	0.0748	533	1.0998	0.983
c1 26221	0.0809	0.0812	993	1.9002	1.9278
g8 26369	0.0947	0.0941	c1 26220	0.0777	0.0773
f13 19503	0.0758	0.0779	w1 29017	0.0852	0.0891
f1 35413	0.0835	0.0855	il 34957	0.1264	0.1122
e2 29064	0.087	0.086	m3 29542	0.0902	0.0801
sample diluent	0.0534	0.059	sample diluent	0.0539	0.067

sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2
m3 29541	0.0845	0.0864	i2 17849	0.0728	0.0865
i2 6758	0.0668	0.0712	w6 27416	0.1166	0.1081
w6 33279	0.0786	0.0774	w1 27105	0.0866	0.0878
h2 35229	0.0877	0.0861	elms	0.0995	0.099
m1 20108	0.1146	0.0967	peanut 1	0.0703	0.0737
f23 20281	0.0787	0.0761	peanut 2	0.0626	0.0702
i3 16793	0.102	0.0962	peanut 3	0.0779	0.108
m6 29659	0.0844	0.0834	sample diluent	0.0566	0.0605

Attachment A

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date June 11, 2001

From Medical Officer, Epidemiology Team, Division of Market Studies (HFS-728), Office of Scientific Analysis and Support

Subject Method Development for Identification of IgE Antibody against Cry9C Protein in StarLink Corn - Decision not to Test for IgG Antibody

To Record

In mid-October of 2000, the Environmental Protection Agency (EPA) requested FDA's assistance in assessing the significance of adverse event reports possibly linked to StarLink™ corn. FDA, in turn, requested assistance from the Centers for Disease Control and Prevention (CDC) in a collaborative effort to study the possible clinical significance of those reports. The initial results of the effort were presented at the November 28, 2000 meeting of EPA's Scientific Advisory Panel. Also presented by CDC was a proposal for further investigation. The proposal included establishment of a case definition; interviews with, and collection of medical records from, those consumers who met the case definition; and also collection of blood samples from consenting individuals. Blood samples were to be banked, pending development of a serological test to examine them for antibodies to Cry9C protein. (Such a test had not yet been developed at the time of the SAP meeting.) CDC and FDA received strong support from the SAP to pursue this avenue in assisting EPA, in its efforts to resolve questions regarding the potential allergenicity of Cry9C protein. After considering various options, FDA decided to develop the test as an enzyme-linked immunosorbent assay (ELISA) for IgE antibodies to Cry9C protein. This memorandum describes factors considered in deciding to focus on IgE antibodies only.

In the initial stages of designing a method to analyze human serum for antibodies that react with (i.e., bind to) Cry9C protein, FDA gave consideration to analyzing serum samples for both IgE and IgG antibodies. However, re-examination of both the scientific literature (see, for example, Binslev-Jensen and Poulsen, 1997; Sampson 1997; Sampson and Burks, 1996) and comments from members of the FIFRA Scientific Advisory Panel (SAP) from the meeting held November 28, 2000, suggested that analyzing for only IgE was the judicious approach to follow. For example, Sampson, 1997 noted: "The vast majority of immediate allergic reactions to foods are due to IgE-mediated hypersensitivity." This judgement is reflective of the views of most experts in this area. Further, the report of the November 28, 2000 meeting of the SAP specifically recommended: "serum should be obtained from these patients [those reporting adverse reactions] and evaluated for the presence of IgE antibody to Cry9C."

Consequently, during the early stages of protocol development, FDA focused greater attention on the need to analyze serum specimens for IgE against Cry9C than for IgG. With EPA agreement,

FDA and CDC held further discussions with selected SAP members who have expertise in immunology and food allergenicity, to seek their advice and determine whether this focus was advisable. The results of these discussions are summarized below.

In a February 20, 2001 meeting at the National Institutes of Health (NIH) with Dean Metcalfe, M.D. (NIH) met with Carol Rubin, DVM (CDC), Robert Vogt, Ph.D. (CDC), Richard Raybourne, Ph.D. (FDA), Kristina Williams, Ph.D., (FDA), and Karl Klontz, M.D. (FDA). FDA/CDC described to Dr. Metcalfe the proposed protocol for testing for antibodies to Cry9C protein, which at that time included the possibility of testing for IgG, and asked his advice regarding inclusion of IgG. Dr. Metcalfe suggested that the method be restricted to analyzing for only IgE antibody and provided the following rationale for this opinion: 1) it is IgE antibody that plays the critical clinical role in the genesis of true allergic reactions from foods, not IgG; and 2) there is a real possibility that cross-reacting IgG antibodies (IgG antibodies that react to more than one protein) could cloud the picture and add confusion as to the meaning of the IgG.

In late February and early March 2001, FDA and CDC independently reviewed scientific literature, took into account the preponderance of comments at the SAP meeting, and also considered the specific advice from Dr. Metcalfe. Ultimately, CDC decided to request that sera be tested only for IgE reactivity with Cry9C protein because IgE is the only type of antibody that causes immediate hypersensitivity in humans, and that any other antibody reactivity (IgG or IgA) would be irrelevant to the immediate-type allergic reactions specified in CDC's case definition.[a] Put in another way, any results of evaluations for IgG reactivity with Cry9C protein would not be clinically relevant to the case reports. (Affirmed in personal communications, R. Vogt and C. Rubin, CDC, and R. Raybourne, (FDA), March 13, 2001.) Consequently, the FDA focused its efforts exclusively on development of a method to detect IgE antibodies.

Personal communications between Marc Rothenberg M.D. Ph.D. (Allergy and Clinical Immunology, University of Cincinnati) and Richard Raybourne, Ph.D. provided additional support for the approach taken by CDC and FDA. Specifically, Dr. Rothenberg commented that the significance of IgG anti-dietary antigens is uncertain and that most studies have shown that this is a normal response. Further, that if Cry9C protein were shown to be immunoreactive (e.g., induces IgG production) but not allergenic (e.g., no IgE induction) that this may create a problem with interpretation and communication of test results. Consistent with this view, the results of a double-blind, placebo-controlled study (Morgan et al. 1990) of persons with shrimp-specific IgG subclass antibodies showed that while some shrimp-specific IgG subclass levels were increased in shrimp-sensitive subjects, none of the subclass responses were significantly predictive of a positive response to double blind placebo-controlled challenge and therefore were

---

[a] CDC's case definition: An adverse health event which occurred 1 July through 30 November 2000, temporally linking corn product consumption to anaphylactic symptoms within 1 hour of eating, dermatologic symptoms within 12 hours of eating, or gastrointestinal symptoms within 12 hours of eating. Excluded were those cases with gastrointestinal symptoms if more than one member of the group experienced similar symptoms after a common meal, considering these more likely due to an infectious or toxic foodborne etiology than to a food allergic reaction. (Winterton et al., 2001, EIS meeting presentation slides).

not diagnostic of shrimp intolerance.

Based on all of the information and advice described above, FDA focused its method development efforts on detecting anti-Cry9C IgE. Ultimately, CDC requested that FDA test the case samples and controls provided by CDC for IgE antibodies, only.

References

Binslev-Jensen, C. and L.K. Poulsen. 1997. *In vitro* diagnostic methods in the evaluation of food hypersensitivity, Chapter 7, pp. 137-150. In: Food Allergy: Adverse Reactions to Foods and Food additives, Second edition. Metcalfe, D.D., H.A. Sampson, and R.A., Simon (editors). Blackwell Science, Cambridge, MA.

Morgan, J.E., Daul C.B, Lehrer, S.B. 1990. The relationships among shrimp-specific IgG subclass antibodies and immediate adverse reactions to shrimp challenge. *Journal of Allergy and Clinical Immunology*. 86:387-392.

Sampson, H.A. 1997. Immediate reactions to foods in infants and children. Chapter 9, pp.169-182. In: Food Allergy: Adverse Reactions to Foods and Food additives, Second edition. Metcalfe, D.D., H.A. Sampson, and R.A., Simon (editors). Blackwell Science, Cambridge, MA.

Sampson, H.A., and A. W. Burks. 1996. Mechanisms of food allergy. Annu. Rev. Nutr. 15:161-177.

Karl C. Klontz, MD

Karl C. Klontz, M.D.
Medical Officer

Attachment B

PROCEDURE FOR ELISA DETECTION OF IgE ANTIBODIES TO CRY9C

COAT ELISA PLATES

1) Suspend purified Cry9C solution (supplied by Aventis Crop Science) to a concentration of 2 ug/ml in carbonate/bicarbonate buffer, pH 9.6. Suspend crude grass antigen to a concentration of 40 ug/well, and crude cat antigen to a concentration of 0.2 units/ml. (optimum concentration of antigen previous determined by block titration with known positive and negative goat antiserum, supplied by Aventis Crop Science)

2) Pipet 100 ul/well into Dynatech Immulon I plates. Include grass and cat antigen to serve as reagent controls.

3) Incubate overnight at 4C.

ADD SERA TO PLATES

4) Allow plates to equilibrate to RT.

5) Aspirate liquid from wells with 12-channel manifold. Wash plates 1X with phosphate buffered saline (PBS).

6) Block for 2 hrs. RT with PBS-10% heat inactivated fetal bovine serum (HIFBS), 100 ul/well.

7) Aspirate liquid from wells as above. Wash plates 2x with PBS.

8) Dilute sera 1:2 with sample diluent (PBS-5% HIFBS, 0.05% Tween 20). Dilute goat antisera 1:5000 with sample diluent.

9) Add diluted sera to wells, 100 ul/well, in duplicate. Pipet known positive sera from cat and grass allergic individuals, diluted 1:2, into cat and grass antigen-coated wells. Sera provided by IBT reference labs.

10) Incubate plates at RT for 2 hrs. or overnight at 4C.

ADD CONJUGATE

11) Allow plates to equilibrate to RT (if incubated at 4C).

12) Aspirate liquid from wells. Wash plates 4x with PBS-0.1% Tween-20 (wash buffer). Allow wash buffer to remain in wells for 1 min.

13) Add affinity purified peroxidase-conjugated goat anti-human IgE (KPL, Cat. #074-1002) to wells, 100 ul/well. For wells with goat serum, add affinity purified peroxidase-conjugated donkey anti-goat IgG (Jackson Labs, Cat. # 705-035-147). Appropriate dilution of conjugate must be determined for each new lot. Typical dilution is 1:2000 Conjugate is diluted in PBS-10% HIFBS.

14) Incubate 2 hrs. at RT.

DEVELOP COLOR

15) Aspirate liquid from wells and wash 4x as above with PBS-0.1% Tween 20.

16) Add 100 ul/well of substrate solution (TMB-Elisa, Gibco Labs, Cat. #15980-014).

17) Incubate 15 min. at RT.

18) Stop reaction with 100 ul/well 1N H_2SO_4.

19) Read absorbance with 96-well Elisa plate reader, 450 nm.

Attachment C

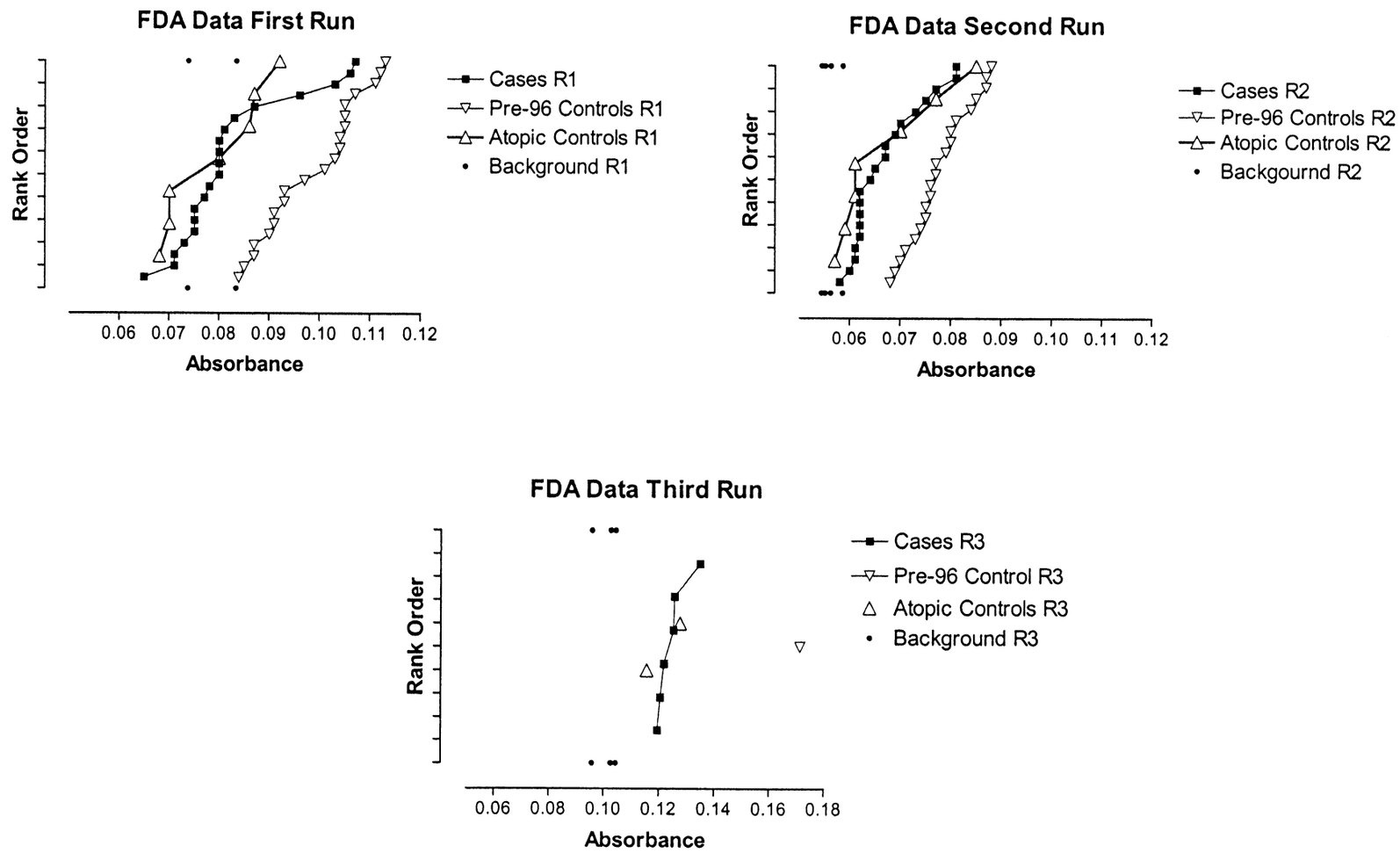
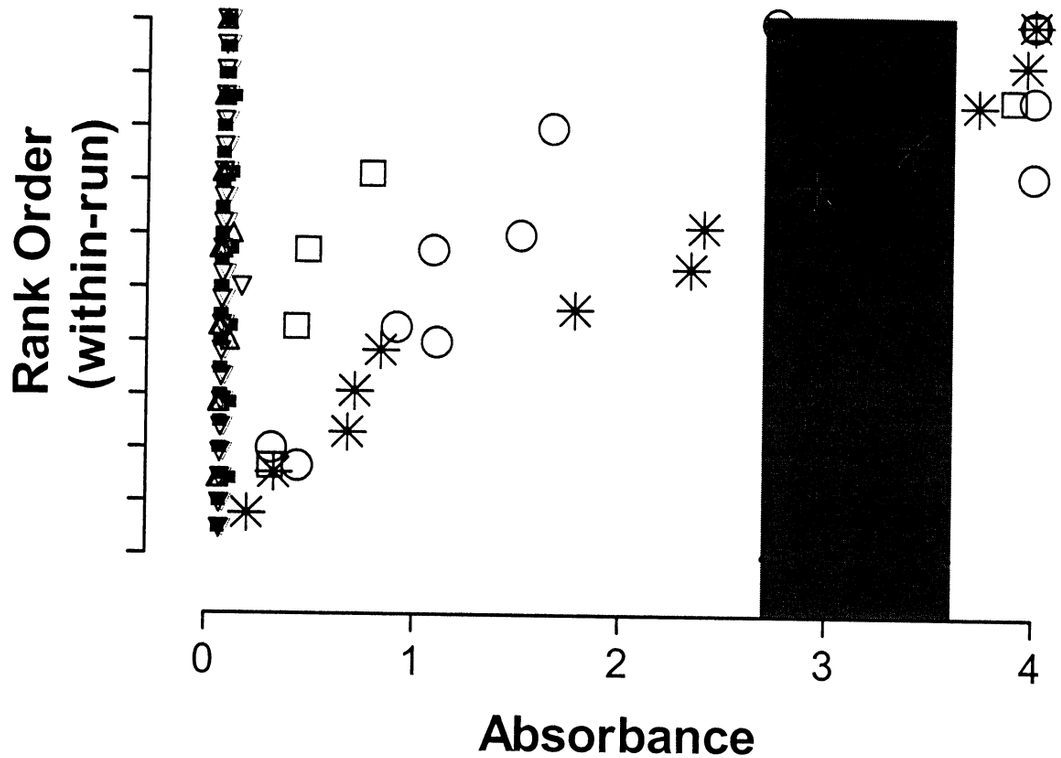


Figure 1

Attachment D



Cry9c – IgE Reactions

- ▪ Cases
- ▽ Pre-96 Controls
- △ Atopic Controls

Allergen – IgE Reactions

- ○ Grass Pos Controls
- □ Cat Pos Controls
- ✳ Peanut Pos Controls

■ **Hyperimmune anti-Cry9c Goat Serum**
(Overall Range for All Runs)

Figure 2

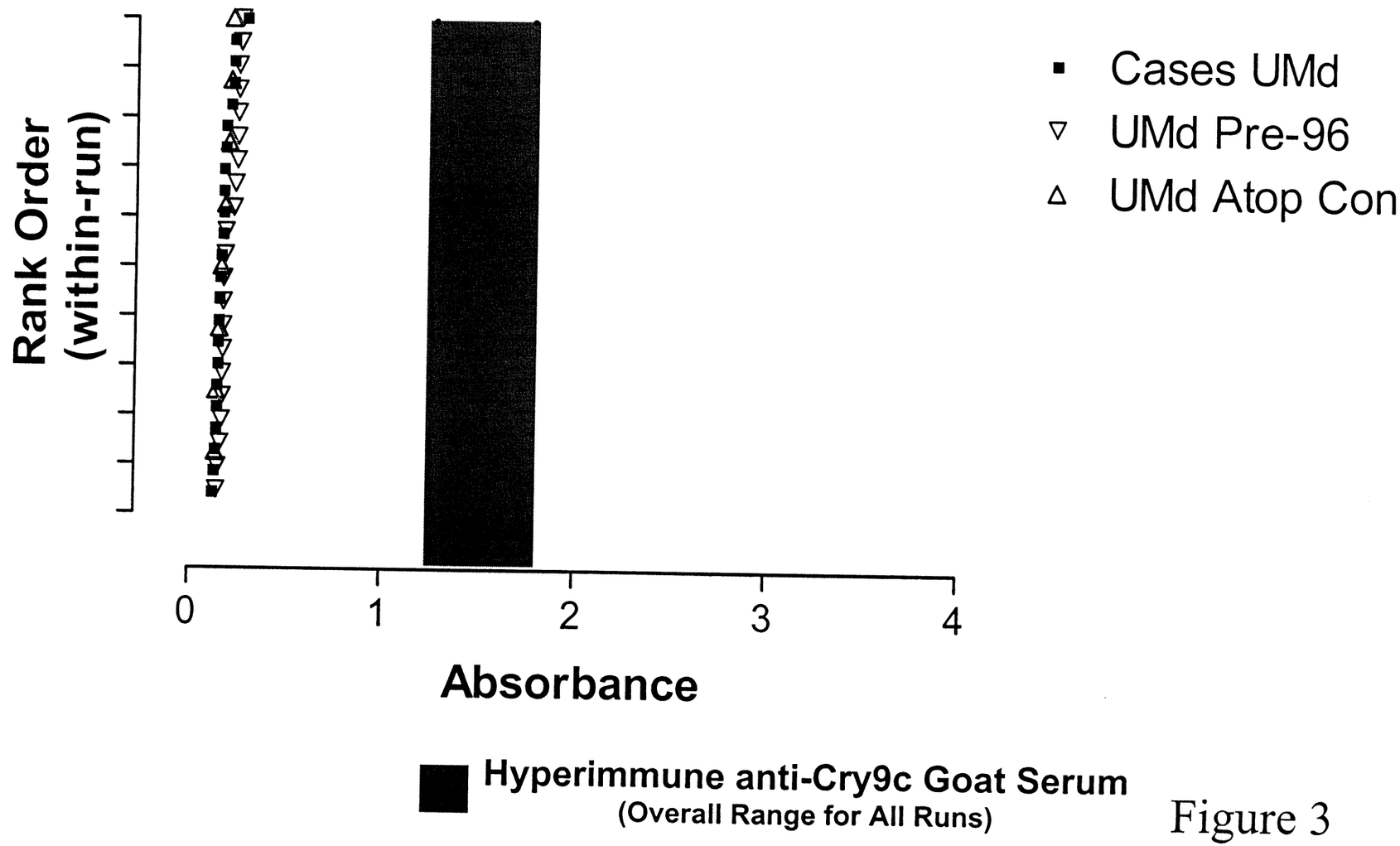

Rank Order
(within-run)
0
1
2
3
4
Absorbance
Cases UMd
UMd Pre-96
UMd Atop Con
Hyperimmune anti-Cry9c Goat Serum
(Overall Range for All Runs)

Figure 3

Attachment F

Date: 5/23/01

From: **Richard B. Raybourne, Ph.D.**
IB, DVA, OARSA, CFSAN, FDA

To: **Dr. Carol Rubin, CDC**

Subject: **ELISA results for human serum samples sent by CDC**

This is in response to your earlier request to analyze the coded samples provided to FDA using our experimental method designed to detect IgE antibody that binds to Cry9c.

Attached is a brief description of the method, which we consider to be still under development. Please note that no human serum known to contain IgE against Cry9c was available for use as a positive control. Included in the test protocol (attached) was a control antiserum against Cry9c raised in goats that assured that Cry9c protein was bound to the ELISA plate wells. To confirm reactivity of the anti-human IgE peroxidase conjugate in the presence of bound IgE, some wells coated with reference standards of cat and grass allergens were included on the plates and tested with known allergic sera to those antigens. In order to maximize the possibility of detecting Cry9c-specific IgE, all samples from CDC were run in duplicate at a 1:2 dilution.

The data provided on the enclosed disk was produced in the FDA laboratory and consists of duplicate optical density readings from the ELISA plate reader for all of the coded samples. A hardcopy of the electronic file is also provided for convenience. Also included are readings from control wells to which sample diluent without serum was added (diluent blank). These readings should be useful in allowing you to determine the inherent background in the system, and to provide a baseline against which to gauge the absolute reactivity of the coded samples. We can also supply the readings for the control sera mentioned above, if you wish.

To provide assurance of reproducibility, we have had the testing confirmed by an independent laboratory at the University of Maryland. We would be very happy to provide you with these data as well.

Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1
0.0858	0.0885	9	0.088	0.096	17	0.0876
0.0828	0.0862	10	0.0909	0.0914	18	0.0855
0.0694	0.0723	11	0.1039	0.0987	19	0.1052
0.066	0.0641	12	0.0675	0.0716	20	0.0864
0.102	0.106	13	0.1016	0.1223	21	0.088
0.075	0.0745	14	0.0948	0.0921	22	0.1066
0.0718	0.0776	15	0.0734	0.0731	23	0.1015
0.1099	0.102	16	0.0758	0.0746	24	0.078

Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1
0.0912	0.1008	41	0.0684	0.0675	49	0.0788
0.0849	0.0867	42	0.0909	0.0824	50	0.079
0.0891	0.0853	43	0.1048	0.1046	297	0.1136
0.068	0.0734	44	0.0804	0.0822	394	0.0816
0.095	0.1188	45	0.0752	0.0815	532	0.1128
0.1042	0.109	46	0.0852	0.0801	534	0.1146
0.0988	0.1108	47	0.1143	0.1079	804	0.135
0.0782	0.0767	48	0.0826	0.078	843	0.143

Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2
0.0979	25	0.0699	0.0724	neg. pool	0.0827	0.0817
0.0892	26	0.1014	0.1049	neg nordlee	0.0665	0.0704
0.1043	27	0.089	0.0926	DD	0.1179	0.1102
0.0817	28	0.0695	0.0697	TK	0.0871	0.0792
0.0923	29	0.0818	0.0782	DV	0.0896	0.095
0.1193	30	0.1039	0.102	o1 1	0.0881	0.1028
0.107	31	0.0933	0.1009	TB	0.1306	0.126
0.0826	32	0.112	0.0969	sample diluent	0.0834	0.0834

Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2
0.082	sample diluent	0.0784	0.0691
0.0811			
0.1056			
0.0874			
0.1093			
0.1015			
0.1248			
0.1398			

2

##BLOCKS= 2

Plate:	Plate#1			Cry9c	Cry9c	
	Temperature(°C)	sample		OD 1	OD 2	sample
	24.8	normal goat		0.0863	0.1029	1
		immune goat		3.7947	3.6486	2
		elms		0.0928	0.0993	3
			131	0.113	0.1009	4
			132	0.0844	0.0774	5
			281	0.0821	0.0911	6
			533	0.0754	0.0807	7
			993	0.0804	0.0829	8

~End

Plate:	Plate#2			Cry9c	Cry9c	
	Temperature(°C)	sample		OD 1	OD 2	sample
	24.4	normal goat		0.0802	0.0813	33
		immune goat		3.6932	3.5897	34
		control 9		0.0739	0.0766	35
			255	0.0666	0.0716	36
			315	0.0914	0.0939	37
			321	0.0897	0.0847	38
			661	0.0899	0.0936	39
			991	0.1257	0.1344	40

~End

##BLOCKS= 3

Plate: Plate#1

Temperature(°C)	sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1
24.5	normal goat	0.0706	0.0648	1	0.0675
	immune goat	2.7486	2.8164	2	0.0761
	9	0.0635	0.0608	3	0.062
	255	0.0627	0.059	4	0.0604
	315	0.0722	0.0692	5	0.0792
	321	0.0666	0.0642	6	0.0617
	661	0.0747	0.0729	7	0.0606
	991	0.0821	0.0817	8	0.0823

~End

Plate: Plate#2

Temperature(°C)	sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1
24.5	normal goat	0.0729	0.0724	32	0.0764
	immune goat	2.8934	2.9091	33	0.0795
	297	0.0885	0.087	34	0.0767
	394	0.0667	0.0754	35	0.0841
	532	0.0935	0.0876	36	0.0637
	534	0.0787	0.0716	37	0.0684
	804	0.1201	0.1079	38	0.0843
	843	0.1034	0.0963	39	0.0862

~End

Plate: Plate#3

Temperature(°C)	sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1
24.6	normal goat	0.0828	0.0766	m3 34965	0.0857
	immune goat	3.4272	3.4782	i1 28054	0.0864
	w10 18012	0.0812	0.0813	m5 29661	0.089
	m5 29654	0.1127	0.0945	e4 13891/2	0.0962
	e3 15521	0.0926	0.0864	f13 33295	0.0893
	f23 13379	0.1119	0.094	f1 19245	0.0949
	d1 35018	0.0881	0.083	f23 26224	0.0885
	e2 29065	0.0775	0.0812	d2 28555	0.0833

~End

4

Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2
0.0674	9	0.0752	0.0944	17	0.0726	0.0759
0.073	10	0.079	0.0728	18	0.0734	0.0675
0.0636	11	0.095	0.0784	19	0.0789	0.0745
0.0613	12	0.0599	0.059	20	0.0678	0.0672
0.0803	13	0.0812	0.0783	21	0.0759	0.0697
0.0619	14	0.0686	0.0688	22	0.075	0.0765
0.0618	15	0.0597	0.0571	23	0.0777	0.0732
0.0806	16	0.0625	0.0609	24	0.0654	0.0632

Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2
0.0776	40	0.0626	0.0613	48	0.0593	0.0625
0.0743	41	0.0612	0.0616	49	0.0703	0.0791
0.0769	42	0.0745	0.0759	50	0.0699	0.0758
0.0769	43	0.0853	0.0893	h2 27609	0.0806	0.0803
0.0668	44	0.0715	0.0689	g6 26589	0.0728	0.0695
0.0692	45	0.0619	0.0628	f2 29663	0.0843	0.084
0.0835	46	0.0624	0.0612	e1 26504	0.0955	0.0957
0.0835	47	0.0858	0.0904	t7 28312	0.0829	0.0765

Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2
0.089	i3 34965	0.0854	0.0788	m6 29396	0.0868	0.0887
0.0913	e2 161841/3	0.0969	0.1085	m1 33333	0.093	0.0845
0.0898	w10 28445	0.1009	0.0986	e4 18922	0.0742	0.071
0.1098	e3 27814	0.0852	0.0839	e4 16530	0.0916	0.0885
0.0969	w10 35390	0.0791	0.0769	f1 29600	0.0787	0.0768
0.0941	h2 26737	0.0839	0.0903	d2 35019	0.0912	0.0872
0.0894	d1 7569	0.0923	0.0851	e3 14338	0.0719	0.0708
0.0844	e1 27623	0.0977	0.1	i1 6763	0.0987	0.0965

sample	Cry9c OD 1	Cry9c OD 2	sample	grass OD 1	grass OD 2
25	0.0604	0.0591	131	2.7211	2.7853
26	0.0819	0.077	132	1.604	1.7301
27	0.0697	0.07	g6 27613	0.3029	0.3319
28	0.0546	0.0586	g8 14330	1.0812	1.1405
29	0.0672	0.0661	g6 24590	1.5446	1.4853
30	0.0838	0.0786	f13 35234	0.1753	0.1618
31	0.0702	0.0721	d2 25570	1.5198	1.3396
sample diluent	0.0554	0.0533	sample diluent	0.054	0.0561

sample	Cry9c OD 1	Cry9c OD 2	sample	cat OD 1	cat OD 2
m5 29633	0.0808	0.0792	281	1.7544	1.6966
c1 26218	0.0735	0.0748	533	1.0998	0.983
c1 26221	0.0809	0.0812	993	1.9002	1.9278
g8 26369	0.0947	0.0941	c1 26220	0.0777	0.0773
f13 19503	0.0758	0.0779	w1 29017	0.0852	0.0891
f1 35413	0.0835	0.0855	il 34957	0.1264	0.1122
e2 29064	0.087	0.086	m3 29542	0.0902	0.0801
sample diluent	0.0534	0.059	sample diluent	0.0539	0.067

sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2
m3 29541	0.0845	0.0864	i2 17849	0.0728	0.0865
i2 6758	0.0668	0.0712	w6 27416	0.1166	0.1081
w6 33279	0.0786	0.0774	w1 27105	0.0866	0.0878
h2 35229	0.0877	0.0861	elms	0.0995	0.099
m1 20108	0.1146	0.0967	peanut 1	0.0703	0.0737
f23 20281	0.0787	0.0761	peanut 2	0.0626	0.0702
i3 16793	0.102	0.0962	peanut 3	0.0779	0.108
m6 29659	0.0844	0.0834	sample diluent	0.0566	0.0605

##BLOCKS= 1

Plate:	Plate#1		Cry9c	Cry9c		Cry9c
	Temperature(°C)	sample	OD 1	OD 2	sample	OD 1
	24.3	normal goat	0.1379	0.1237	47	0.1691
		immune goat	4	4	48	0.1325
		51	0.1392	0.1306	49	0.1149
		52	0.1297	0.1255	50	0.1257
		53	0.1297	0.1138	d2 25570	0.1453
		54	0.1213	0.1196	w1 29017	0.1416
		45	0.1103	0.1285	i1 34957	0.1478
		46	0.1271	0.1451	m3 29542	0.1477

~End

Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2	sample	Cry9c OD 1	Cry9c OD 2
0.1734	i2 13378	0.1871	0.1972	nhp7	0.1269	0.1329
0.0987	w6 16752	0.1587	0.1576	nhp8	0.0975	0.1007
0.1356	nhp1	0.1084	0.1018	nhp9	0.1172	0.1427
0.1256	nhp2	0.1285	0.1567	nhp10	0.0977	0.0902
0.1276	nhp3	0.1117	0.1138	nhp11	0.1179	0.1044
0.1419	nhp4	0.1475	0.099	nhp12	0.0983	0.1002
0.1259	nhp5	0.1127	0.1095	nhp13	0.104	0.0952
0.1364	nhp6	0.1314	0.1453	sample diluent	0.0955	0.096

sample	cat	cat	sample	grass	grass
281	OD 1	OD 2	131	OD 1	OD 2
533	3.978	3.8142	132	3.9921	4
993	4	4	g6 27613	1.0433	1.1381
e1 26504	0.307	0.3187	g6 26589	0.4136	0.4765
e1 26588	0.8518	0.73	g6 29590	4	4
e1 27623	0.5021	0.4714	g8 14330	4	4
h2 27609	0.3995	0.4694	g8 26396	0.902	0.9273
sample diluent	0.0938	0.1114	sample diluent	0.1045	0.1042

##BLOCKS= 6

Plate: Plate#1 1.1 PlateForma Endpoint Absorbance Raw 1

Temperatu	1	2	3	4	5	6	7
	Neg 1	Neg 1	Neg 2	Neg 2	11	11	JB
1:10	0.1378	0.1212	0.1508	0.1541	0.3336	0.3313	0.6876
	0.1041	0.1052	0.1258	0.1241	0.2224	0.2067	0.4323
	0.0848	0.0852	0.1022	0.1053	0.1496	0.1524	0.3003
	0.0738	0.0765	0.0883	0.0831	0.1115	0.1124	0.1946
	0.0638	0.0693	0.0739	0.0732	0.0933	0.0906	0.1378
	0.0611	0.0612	0.0654	0.0664	0.0787	0.0796	0.1116
	0.0622	0.0574	0.0617	0.063	0.0732	0.0714	0.0883
	0.0692	0.0615	0.0601	1.5198	0.0674	0.0645	0.0735

~End

Plate: Plate#3 1.1 PlateForma Endpoint Absorbance Raw 1

Temperatu	1	2	3	4	5	6	7
	Pea 3	Pea 3	19503	19503	33295	33295	35234
1:10	3.4213	3.4209	0.2071	0.2022	0.7603	0.6747	2.2826
	2.5763	2.3973	0.1773	0.1734	0.384	0.3848	1.1738
	1.8334	1.7137	0.1487	0.1482	0.2349	0.2346	0.6382
	1.1742	1.1456	0.1179	0.1195	0.1529	0.1505	0.3673
	0.7402	0.6858	0.0924	0.0923	0.1055	0.1041	0.2162
	0.4557	0.4252	0.1425	0.075	0.085	0.0821	0.1438
	0.2644	0.2619	0.0671	0.0681	0.0719	0.0711	0.1022
	0.1965	0.1924	0.0667	0.0663	0.0658	0.0638	0.0862

~End

Plate: Plate#5 1.1 PlateForma Endpoint Absorbance Raw 1

Temperatu	1	2	3	4	5	6	7
	SM	SM	DV	DV	DS	DS	DD
1:10	1.9027	1.661	4	4	0.8244	0.5462	4
	1.2579	1.2651	3.5712	3.5461	0.2733	0.2696	3.6762
	0.7863	0.7938	2.6374	2.8148	0.1696	0.161	3.4897
	0.609	0.5562	1.768	1.7738	0.1143	0.116	2.4154
	0.3996	0.381	1.0693	1.0246	0.0981	0.0941	1.451
	0.2529	0.2684	0.6884	0.6335	0.0882	0.0828	0.8834
	0.1695	0.1796	0.3794	0.3668	0.0762	0.0731	0.5221
	0.1224	0.1265	0.2447	0.2204	0.0685	0.0676	0.3235

~End

10

1 450 1 12

8	9	10	11	12
JB	Pea 1	Pea 1	Pea 2	Pea2
0.7306	3.6988	3.7587	2.4637	2.3461
0.4467	3.2457	3.1553	1.5601	1.5194
0.3112	2.1026	2.1081	0.9259	0.9201
0.2109	1.3866	1.3179	0.5098	0.5511
0.1494	0.8177	0.8333	0.3198	0.3212
0.1101	0.4985	0.5011	0.2106	0.2064
0.0935	0.3552	0.319	0.1373	0.1471
0.0763	0.2206	0.2169	0.1026	0.1115

1 450 1 12

8	9	10	11	12
35234 GH	GH	TK	TK	
2.4048	2.9059	2.9781	0.8623	0.8194
1.2403	1.7414	1.7462	0.4678	0.476
0.6294	1.029	1.055	0.3286	0.3458
0.3586	0.6286	0.6282	0.2117	0.2448
0.2131	0.3997	0.4079	0.1449	0.1652
0.1392	0.2641	0.2697	0.109	0.1195
0.1021	0.1692	0.1807	0.0934	0.1019
0.0805	0.1213	0.1323	0.0858	0.096

1 450 1 12

8	9	10	11	12
DD				
3.9226	0.0384	0.0411	0.041	0.0402
3.6029	0.0376	0.0378	0.0483	0.045
3.2452	0.0377	0.0484	0.0461	0.0515
2.4774	0.0376	0.0384	0.0494	0.055
1.5136	0.0376	0.0379	0.0368	0.0371
1.0109	0.0386	0.0384	0.0388	0.0433
0.6176	0.0374	0.0373	0.0378	0.039
0.3787	0.0382	0.0396	0.0364	0.0371

1 450 1 12

11

Attachment G

Date: **5/24/01**

From: **Richard B. Raybourne, Ph.D.**
IB, DVA, OARSA, CFSAN, FDA

To: **Dr. Carol Rubin, CDC**

Subject: **ELISA results from University of Maryland**

Per earlier communications, attached are the data, on disk and hardcopy obtained by an independent laboratory at the University of Maryland using the coded serum samples provided by CDC and FDA's ELISA method for detection of human IgE (protocol supplied as an attachment to my memo dated 5/23/01). Please note that the data labeled "Blank Correction" have the OD value for sample diluent subtracted out.

FDA Cry9C Study

PLATE 1

	OD 1	OD 2	Avg OD	Std Dev	Blank Correction
CAT					
281	1.365	1.391	1.378	0.018385	1.255
26588	1.759	1.352	1.5555	0.287792	1.4325
26504	0.953	1.074	1.0135	0.08556	0.8905
27623	1.279	1.315	1.297	0.025456	1.174
27609	1.6	1.238	1.419	0.255973	1.296
NHP-1	0.26	0.228	0.244	0.022627	0.121
NHP-2	0.251	0.245	0.248	0.004243	0.125
Blank	0.122	0.124	0.123	0.001414	0

	OD 1	OD 2	Avg OD	Std Dev	Blank Correction
GRASS					
26589	0.267	0.31	0.2885	0.030406	0.169
14330	1.1	1.309	1.2045	0.147785	1.085
29590	1.411	1.45	1.4305	0.027577	1.311
27613	1.845	1.727	1.786	0.083439	1.6665
132	1.422	1.425	1.4235	0.002121	1.304
131	1.495	1.565	1.53	0.049497	1.4105
NHP-10	0.321	0.287	0.304	0.024042	0.1845
Blank	0.112	0.127	0.1195	0.010607	0

	OD 1	OD 2	OD 3	OD 4	Avg OD	Std Dev	Blank Corr
Anti Cry9C	1.559	1.55	1.778	1.579	1.6165	0.108346666	1.3595
Normal Goat	0.242	0.296	0.273	0.261	0.268	0.022612681	0.011
anti Goat	0.25	0.264			0.257	0.009899495	0
anti Human	0.139	0.116			0.1275	0.016263456	0.05925
Blank	0.076	0.06	0.075	0.062	0.06825	0.008421203	0

SAMPLE #	OD 1	OD 2	Avg OD	Std Dev	Blank Correction
1	0.203	0.206	0.2045	0.002121	0.077
2	0.157	0.134	0.1455	0.016263	0.018
3	0.13	0.127	0.1285	0.002121	0.001
4	0.127	0.151	0.139	0.016971	0.0115
5	0.169	0.133	0.151	0.025456	0.0235
6	0.136	0.168	0.152	0.022627	0.0245
7	0.176	0.133	0.1545	0.030406	0.027
8	0.164	0.182	0.173	0.012728	0.0455
9	0.172	0.19	0.181	0.012728	0.0535
10	0.17	0.18	0.175	0.007071	0.0475
11	0.216	0.135	0.1755	0.057276	0.048
12	0.169	0.156	0.1625	0.009192	0.035
13	0.159	0.165	0.162	0.004243	0.0345
14	0.192	0.153	0.1725	0.027577	0.045
15	0.156	0.187	0.1715	0.02192	0.044
16	0.17	0.147	0.1585	0.016263	0.031
17	0.254	0.23	0.242	0.016971	0.1145
18	0.203	0.222	0.2125	0.013435	0.085
19	0.273	0.249	0.261	0.016971	0.1335
20	0.262	0.248	0.255	0.009899	0.1275
21	0.231	0.22	0.2255	0.007778	0.098
22	0.246	0.22	0.233	0.018385	0.1055
23	0.255	0.224	0.2395	0.02192	0.112
24	0.24	0.206	0.223	0.024042	0.0955

		FDA Cry9C Study							
		PLATE 2							
			OD 1	OD 2	Avg OD	Std Dev	Blank Correction		
		CAT							
		281	1.228	1.509	1.3685	0.198697	1.259		
		26588	1.312	1.062	1.187	0.176777	1.0775		
		26504	1.111	1.16	1.1355	0.034648	1.026		
		27623	1.559	1.465	1.512	0.066468	1.4025		
		27609	1.775	1.51	1.6425	0.187383	1.533		
		NHP-1	0.249	0.246	0.2475	0.002121	0.138		
		NHP-2	0.273	0.244	0.2585	0.020506	0.149		
		Blank	0.111	0.108	0.1095	0.002121	0		
			OD 1	OD 2	Avg OD	Std Dev	Blank Correction		
		GRASS							
		26589	0.274	0.285	0.2795	0.007778	0.1585		
		14330	1.721	1.499	1.61	0.156978	1.489		
		29590	>2.0	>2.0	>2.0	?	?		
		27613	1.807	1.787	1.797	0.014142	1.676		
		132	1.39	1.609	1.4995	0.154856	1.3785		
		131	1.473	1.434	1.4535	0.027577	1.3325		
		NHP-10	0.295	0.322	0.3085	0.019092	0.1875		
		Blank	0.111	0.131	0.121	0.014142	0		
ection			OD 1	OD 2	OD 3	OD 4	Avg OD	Std Dev	Blank Corr
		Anti Cry9C	1.361	1.488	1.58	1.58	1.50225	0.103674	1.22075
		Normal Goat	0.308	0.285	0.259	0.211	0.26575	0.041628	-0.01575
		anti Goat	0.292	0.271			0.2815	0.014849	0
		anti Human	0.138	0.117			0.1275	0.014849	0.0445
		Blank	0.088	0.069	0.11	0.065	0.083	0.020607	0
		SAMPLE #	OD 1	OD 2	Avg OD	Std Dev	Blank Correction		
		25	0.192	0.166	0.179	0.018385	0.0515		
		26	0.25	0.244	0.247	0.004243	0.1195		
		27	0.199	0.165	0.182	0.024042	0.0545		
		28	0.156	0.121	0.1385	0.024749	0.011		
		29	0.141	0.145	0.143	0.002828	0.0155		
		30	0.152	0.173	0.1625	0.014849	0.035		
		31	0.188	0.164	0.176	0.016971	0.0485		
		32	0.168	0.177	0.1725	0.006364	0.045		
		33	0.152	0.142	0.147	0.007071	0.0195		
		34	0.143	0.138	0.1405	0.003536	0.013		
		35	0.17	0.199	0.1845	0.020506	0.057		
		36	0.165	0.175	0.17	0.007071	0.0425		
		37	0.129	0.141	0.135	0.008485	0.0075		
		38	0.184	0.165	0.1745	0.013435	0.047		
		39	0.161	0.181	0.171	0.014142	0.0435		
		40	0.132	0.122	0.127	0.007071	-0.0005		
		41	0.222	0.192	0.207	0.021213	0.0795		
		42	0.248	0.243	0.2455	0.003536	0.118		
		43	0.268	0.222	0.245	0.032527	0.1175		
		44	0.224	0.215	0.2195	0.006364	0.092		
		45	0.238	0.2	0.219	0.02687	0.0915		
		46	0.295	0.279	0.287	0.011314	0.1595		
		47	0.253	0.233	0.243	0.014142	0.1155		
		48	0.226	0.166	0.196	0.042426	0.0685		

		FDA Cry9C Study							
		PLATE 3							
			OD 1	OD 2	Avg OD	Std Dev	Blank Correction		
		CAT							
		281	1.857	1.689	1.773	0.118794	1.6845		
		26588	>2.0	1.109	>1.109	?	?		
		26504	1.185	1.141	1.163	0.031113	1.0745		
		27623	1.564	1.522	1.543	0.029698	1.4545		
		27609	1.549	1.282	1.4155	0.188798	1.327		
		NHP-1	0.195	0.185	0.19	0.007071	0.1015		
		NHP-2	0.22	0.154	0.187	0.046669	0.0985		
		Blank	0.084	0.093	0.0885	0.006364	0		
			OD 1	OD 2	Avg OD	Std Dev	Blank Correction		
		GRASS							
		26589	0.243	0.3	0.2715	0.040305	0.164		
		14330	1.492	1.076	1.284	0.294156	1.1765		
		29590	1.311	1.186	1.2485	0.088388	1.141		
		27613	1.145	1.563	1.354	0.295571	1.2465		
		132	1.348	1.443	1.3955	0.067175	1.288		
		131	1.663	1.36	1.5115	0.214253	1.404		
		NHP-10	0.293	0.286	0.2895	0.00495	0.182		
		Blank	0.09	0.125	0.1075	0.024749	0		
ection			OD 1	OD 2	OD 3	OD 4	Avg OD	Std Dev	Blank Corr
		Anti Cry9C	1.291	1.365	1.74	1.274	1.4175	0.218599	1.251
		Normal Goat	0.188	0.188	0.2	0.188	0.191	0.006	0.0245
		anti Goat	0.175	0.158			0.1665	0.012021	0
		anti Human	0.1	0.073			0.0865	0.019092	0.02675
		Blank	0.067	0.05	0.069	0.053	0.05975	0.009639	0
		SAMPLE #	OD 1	OD 2	Avg OD	Std Dev	Blank Correction		
		49	0.137	0.168	0.1525	0.02192	0.066		
		50	0.157	0.143	0.15	0.009899	0.0635		
		2001-45	0.14	0.127	0.1335	0.009192	0.047		
		2001-46	0.205	0.135	0.17	0.049497	0.0835		
		2001-47	0.148	0.122	0.135	0.018385	0.0485		
		2001-48	0.129	0.115	0.122	0.009899	0.0355		
		20001-49	0.183	0.191	0.187	0.005657	0.1005		
		2001-50	0.129	0.126	0.1275	0.002121	0.041		
		#51	0.187	0.176	0.1815	0.007778	0.095		
		#52	0.163	0.147	0.155	0.011314	0.0685		
		#53	0.182	0.163	0.1725	0.013435	0.086		
		#54	0.164	0.128	0.146	0.025456	0.0595		
		804	0.231	0.226	0.2285	0.003536	0.142		
		297	0.208	0.22	0.214	0.008485	0.1275		
		321	0.129	0.118	0.1235	0.007778	0.037		
		532	0.185	0.163	0.174	0.015556	0.0875		
		991	0.188	0.166	0.177	0.015556	0.0905		
		532	0.215	0.219	0.217	0.002828	0.1305		
		29064	0.193	0.177	0.185	0.011314	0.0985		
		18012	0.186	0.208	0.197	0.015556	0.1105		
		13378	0.194	0.188	0.191	0.004243	0.1045		
		16752	0.219	0.265	0.242	0.032527	0.1555		
		35019	0.191	0.208	0.1995	0.012021	0.113		
		14338	0.17	0.157	0.1635	0.009192	0.077		

FDA Cry9C Study

PLATE 4

	OD 1	OD 2	Avg OD	Std Dev	Blank Correction
CAT					
281	1.337	1.21	1.2735	0.089803	1.1855
26588	1.588	1.486	1.537	0.072125	1.449
26504	1.093	1.125	1.109	0.022627	1.021
27623	1.398	1.336	1.367	0.043841	1.279
27609	1.556	1.576	1.566	0.014142	1.478
NHP-1	0.22	0.233	0.2265	0.009192	0.1385
NHP-2	0.23	0.24	0.235	0.007071	0.147
Blank	0.07	0.106	0.088	0.025456	0

	OD 1	OD 2	Avg OD	Std Dev	Blank Correction
GRASS					
26589	0.3	0.299	0.2995	0.000707	0.1845
14330	1.098	1.512	1.305	0.292742	1.19
29590	1.32	1.098	1.209	0.156978	1.094
27613	1.101	1.63	1.3655	0.374059	1.2505
132	1.284	1.529	1.4065	0.173241	1.2915
131	1.311	1.387	1.349	0.05374	1.234
NHP-10	0.293	0.294	0.2935	0.000707	0.1785
Blank	0.116	0.114	0.115	0.001414	0

ection	OD 1	OD 2	OD 3	OD 4	Avg OD	Std Dev	Blank Corr
Anti Cry9C	1.412	1.341	1.545	1.365	1.41575	0.091073	1.16525
Normal Goat	0.24	0.201	0.292	0.213	0.2365	0.040435	-0.014
anti Goat	0.291	0.21			0.2505	0.057276	0
anti Human	0.153	0.108			0.1305	0.03182	0.06025
Blank	0.082	0.055	0.08	0.064	0.07025	0.012971	0

SAMPLE #	OD 1	OD 2	Avg OD	Std Dev	Blank Correction
28054	0.177	0.143	0.16	0.024042	0.0295
27037	0.16	0.134	0.147	0.018385	0.0165
26220	0.169	0.129	0.149	0.028284	0.0185
13379	0.252	0.207	0.2295	0.03182	0.099
26224	0.165	0.149	0.157	0.011314	0.0265
2855	0.214	0.147	0.1805	0.047376	0.05
20108	0.324	0.267	0.2955	0.040305	0.165
20281	0.186	0.148	0.167	0.02687	0.0365
NHP-4	0.156	0.144	0.15	0.008485	0.0195
NHP-3	0.171	0.196	0.1835	0.017678	0.053
NHP-6	0.193	0.169	0.181	0.016971	0.0505
NHP-5	0.129	0.12	0.1245	0.006364	-0.006
NHP-8	0.175	0.169	0.172	0.004243	0.0415
NHP-7	0.193	0.156	0.1745	0.026163	0.044
NHP-9	0.204	0.132	0.168	0.050912	0.0375
NHP-11	0.18	0.157	0.1685	0.016263	0.038
NHP-12	0.216	0.187	0.2015	0.020506	0.071
NHP-13	0.219	0.215	0.217	0.002828	0.0865

4