

MILK

PEANUT

ALMOND

EGG

HAZELNUT

SOY FLOUR

GLIADIN

Food Allergen Handbook

SIXTH EDITION



Includes a
New Q & A
Section



NEOGEN
CORPORATION

food allergy research
& resource program
farrp

Presented by Neogen Corporation in cooperation with the
University of Nebraska's Food Allergy Research & Resource Program (FARRP)

Neogen Corporation develops and markets products and services dedicated to food and animal safety. The Company's Food Safety Division markets diagnostic test kits to detect foodborne bacteria, natural toxins, genetic modifications, food allergens, drug residues, plant diseases and sanitation concerns. These diagnostic test kits are less expensive, easier to use, and provide greater accuracy and speed than many of the conventional diagnostic methods currently employed. Neogen's Acumedia subsidiary has been a premier manufacturer of dehydrated culture media since 1978. For more information, please call 800/234-5333 or 517/372-9200.

The University of Nebraska's Food Allergy Research and Resource Program (FARRP) is a part of the Department of Food Science and Technology and the Food Processing Center. FARRP is a food industry and university partnership which was formed to provide research and resource tools for the food industry in the area of food allergens. It is the leader in training and educating the industry on allergen awareness. For more information, please call 402/472-4430.

TABLE OF CONTENTS

What is food allergy?	2
Why test for food allergens?	2
The food allergen labeling and Consumer Protection Act of 2004	2
Why test for gliadin/gluten?	3
How do rapid tests help prevent allergen cross-contact?	3
Screening vs. quantifying results.....	3
How do Neogen's food allergen tests work?.....	4
Limitations of ELISA-based food allergen tests.....	4
Veratox and Alert food allergen sandwich ELISA tests.....	5
Reveal food allergen screening test	5
Sampling ingredients, products, liquids and rinses.....	6
Extracting allergens from ingredients, products, liquids and rinses.....	6
Extracting gliadin from ingredients, products, liquids and rinses.....	7
Environmental sampling and extraction.....	8
Reveal for food allergen test procedures	10
Screening: The Alert for food allergen test procedure	11
Quantifying: The Veratox for food allergen test procedure.....	12
How can allergen verification fit into a food safety program?	13
Example: Allergen verification in a food safety program.....	14
Food allergen self-evaluation checklist	15
Some recommended test points for validation of allergen control strategies.....	16
Questions and answers regarding food allergen testing	16
Appendix A: The product specifications of Neogen's Veratox food allergen test kits.....	18
Appendix B: The test validation results for Neogen's food allergen test kits	19
Resources.....	20



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WHAT IS FOOD ALLERGY?

An estimated 3.5 to 4% of adults, and 6 to 8% of children, are allergic to foods. More than 10 million people in the United States alone are known to have a food allergy. While researchers have identified more than 160 foods that contain naturally-occurring proteins that have been shown to cause allergic reactions, researchers also estimate that 90% of all food allergic reactions are caused by just eight common foods: peanuts, eggs, milk, soy, wheat, crustaceans, fish and tree nuts (i.e., walnuts, hazelnuts, almonds, cashews, pistachios, pecans, etc.). Peanuts are the leading cause of severe food allergic reactions.

Food allergens are proteins in some foods that can trigger an immune response in allergic individuals. Current experience indicates that an immune response can be triggered by eating a food containing minute quantities of a food allergen, with the specific amounts needed to trigger the response varying from individual to individual.

Once ingested, food allergens can cause a number of symptoms, ranging from mild hives to severe gastrointestinal and respiratory symptoms, including nausea, vomiting, throat swelling, asthma and trouble breathing. The most serious food-allergic reaction is anaphylactic shock, which is a severe shock reaction that can include any of the symptoms previously described, but also includes a dangerous drop in blood pressure and sometimes cardiac arrhythmia. Anaphylactic shock can be life-threatening if not treated immediately.

WHY TEST FOR FOOD ALLERGENS?

Food manufacturers protect those with food allergies by clearly labeling their products with a list of ingredients. Testing for the presence of food allergens ensures food manufacturers that an unlabeled—and potentially dangerous—ingredient did not make its way into a food product.

Testing also can add to, and protect, a company's reputation. Currently, some companies put a precautionary statement like “may contain peanut and peanut products” on the ingredient label, even though there is very little chance the product actually contains any peanut. If testing is done, companies may be able to minimize the use of precautionary labels.

In companies that use push-through product to clean equipment between products, testing can allow the company to determine exactly how much push-through product is necessary to achieve the level of cleanliness necessary for food allergens. Testing can eliminate guesswork, and save product from going to waste or from having to be reworked.

Testing CIP solutions, final product, and certain equipment after the sanitation crew has finished, can identify sources of cross-contact, and also verify cleanliness before changeover.

The most obvious reason for testing is to protect a company from staggering costs. If a product contains undeclared, potentially hazardous allergens, the company would contact the government and initiate a voluntary recall. Product recalls can cost food companies millions.

THE FOOD ALLERGEN LABELING AND CONSUMER PROTECTION ACT OF 2004

The food allergen labeling law that went into effect Jan. 1, 2006, the Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA), requires food manufacturers to have processes in place to reduce or eliminate accidental cross-contact between non-allergenic food and known food allergens (e.g., peanuts, egg, milk, soy, tree nuts, wheat, etc.).

If there is any chance—intentional or not—that a product contains milk-derived protein, for example, the label must read that the product contains “milk”. For those in the food industry with known allergenic food ingredients in their products, the law will simply mean changing their ingredient labels from a less consumer known term like “semolina” to consumer-friendly “wheat”. For others the new law will require a comprehensive investigative survey of all of their products' minor ingredients.

While the use of allergen test kits is not addressed in FALCPA, tests can be valuable tools for assessing the effectiveness of allergen Good Manufacturing Practices (GMPs). FALCPA includes a requirement that the FDA provide a report to Congress, no later than 18 months after date of enactment, advising “whether

GMPs can be used to reduce or eliminate cross-contact of foods with the major food allergens.” By testing environmental swabs, CIP rinses, push-through product, etc., a company can gauge the effectiveness of their sanitation programs. In fact, in a recent survey of major food manufacturers, the use of test kits has become the “standard of care” in sanitation assessment and validation.

WHY TEST FOR GLIADIN/GLUTEN?

Gliadin is an alcohol-soluble protein found in wheat that belongs to a group of proteins called prolamins. Other prolamins include secalin, found in rye, and hordein, found in barley. Gluten consists of two groups of proteins (prolamins and glutelins) that are found in differing amounts in wheat, barley, rye and oats. Since gliadin represents approximately 50% of gluten, a Neogen Veratox result of 10 ppm would correspond to 20 ppm gluten.

Gliadin and other prolamins have been identified as major causal agents in a number of disorders, including wheat allergy and gluten intolerance (celiac disease). Wheat allergy is a specific immune response to a number of wheat proteins, including gliadin, albumin, globulin, and glutenin. Celiac disease is a chronic reaction to gluten proteins that results in the poor absorption of nutrients in the small intestine.

Those with wheat allergy or celiac disease must avoid gluten, and rely upon the correct labeling of food to make appropriate, safe food choices. Testing for the presence of gluten components ensures food manufacturers that an unlabeled—and potentially dangerous—ingredient did not make its way into a food product.

In addition to the wheat allergen implications, food companies labeling a product as *gluten-free* must ensure that their product meets this claim. Currently, The Codex Alimentarius defines gluten-free as less than 200 ppm gluten (100 ppm gliadin), though a new, proposed standard of 20 ppm gluten (10 ppm gliadin) is being considered.

HOW DO RAPID TESTS HELP PREVENT ALLERGEN CROSS-CONTACT?

Rapid food allergen test kits give a company a method of easily determining if its product has been subjected to cross-contact, and an investigative tool to determine how and when the cross-contact occurred. Companies can use the test kits on raw material before it enters production, or on equipment or product at any point throughout the production process. The tests’ flexibility and ease of use allow users to pinpoint and eliminate possible risks for cross-contact.

SCREENING VS. QUANTIFYING RESULTS

Neogen’s rapid tests for the detection of food allergens and gliadin are available in three formats. Neogen’s Reveal allergen screening test is a simple strip test that requires less than 10 minutes following sample extraction. The company’s screening line of microwell tests, Alert, provides easy-to-interpret visual results. Neogen’s line of quantifying tests, Veratox, uses a microwell reader and preset calculations (programmed into the reader or a computer) to determine exact concentrations of target allergens.

A. Screening tests

Neogen’s line of screening tests allow for the rapid determination of the presence of a target food allergen in food product or environmental swabs.

1. **Reveal.** Designed for ease of use, the Reveal test provides positive or negative test results in 5 or 10 minutes at a predetermined level (e.g., 5 ppm). The lateral flow format is ideally suited for quick pre-operational decision making and requires minimal hands-on time and equipment.
2. **Alert.** A simple dropper-bottle microwell test that provides positive or negative results in 30 minutes or less, the Alert test is ideally suited for batching multiple samples.

B. Quantitative tests

Neogen’s Veratox line provides the concentration of a target food allergen in about 30 minutes. Following the test procedure, color changes in the sample wells are compared to the standards in the control wells using a microwell reader. Exact food allergen concentrations in the samples are computed using the comparisons.

HOW DO NEOGEN'S FOOD ALLERGEN TESTS WORK?

A. Veratox and Alert

Neogen's microwell food allergen tests are sandwich enzyme-linked immunoassays (S-ELISAs), and work on the same principle. A target food allergen protein is extracted from samples with a buffered salt solution (40% ethanol for gliadin). Extracted protein is sampled and added to antibody-coated microwells, where it binds to the antibody during an incubation. Any unbound protein is washed away and a second antibody, which is enzyme-labeled conjugate, is added. The conjugate binds to the already bound protein. After a second wash, substrate is added. Color develops as a result of the presence of bound conjugate. Red Stop reagent is added and the color of the resulting solution is observed. Blue color indicates a strong positive. Red color indicates little to no target food allergen.

B. Reveal (peanut)

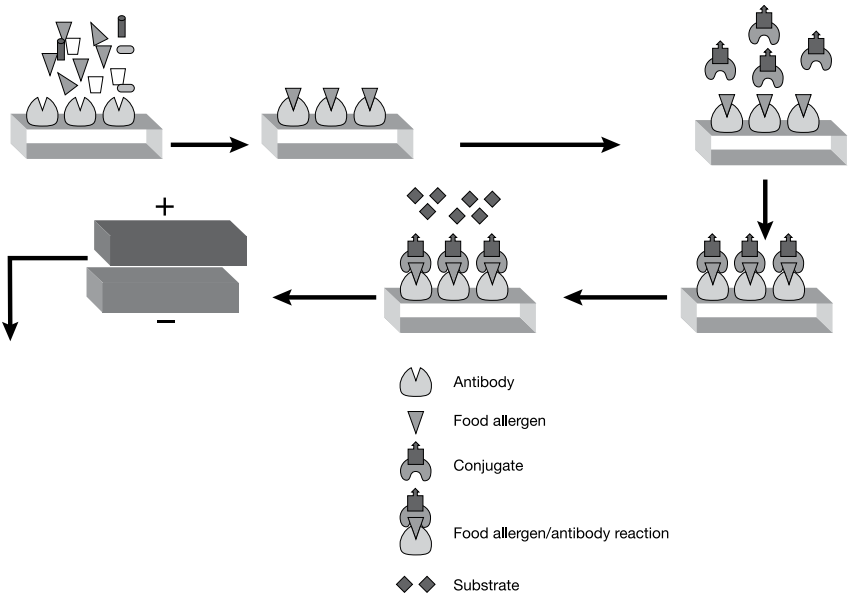
Neogen's Reveal format for the detection of food allergen is a single-step lateral flow immunochromatographic assay. The extract is wicked through a reagent zone, which contains antibodies specific for the target allergen conjugated to colored particles. If allergen is present, it will be captured by the conjugated antibodies. The allergen-antibody-particle complex is then wicked onto a membrane which contains a zone of antibody specific for the target allergen. This zone captures the complex allowing the particles to concentrate and form a visible line. If no target allergen is present, no line will form. The membrane also contains a control zone where an immune complex present in the reagent zone is captured by an antibody, forming a visible line. The control line will always form regardless of the presence of the target allergen, ensuring the strip is working properly.

LIMITATIONS OF ELISA-BASED FOOD ALLERGEN TESTS

ELISA-based food allergen tests, like Neogen's, are not applicable for use in certain applications. Because the tests are based on an antibody reaction with an extracted allergenic protein, the protein in the sample must close to its natural state and readily extractable. Although this is normally the case, in certain instances the test may not yield results totally indicative of the sample's potential to produce an allergic reaction in susceptible consumers. Some of these instances include (but are not limited to):

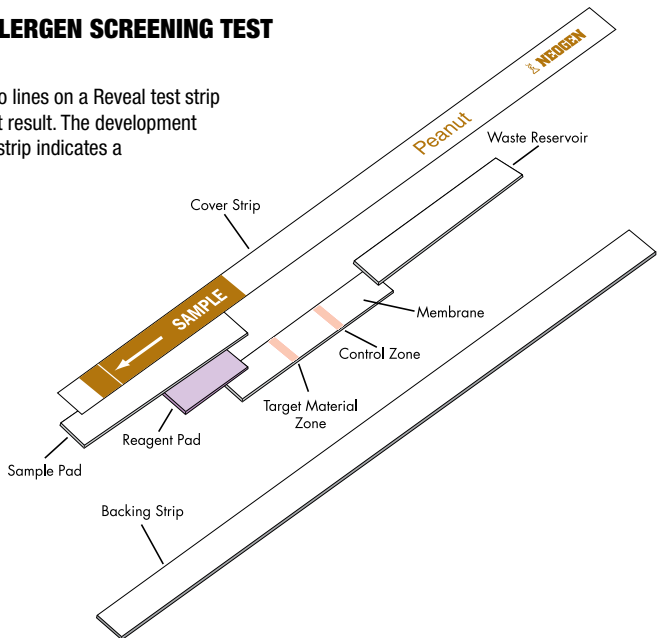
- Hydrolyzed proteins
- Proteolized proteins
- Fermented products and products microbially grown on allergenic substrates
- Probiotic cultures and enzyme preps
- Concentrated food additives, colors and flavors

VERATOX AND ALERT FOOD ALLERGEN SANDWICH ELISA TESTS



REVEAL FOOD ALLERGEN SCREENING TEST

The development of two lines on a Reveal test strip indicates a positive test result. The development of only one line on the strip indicates a negative result.



SAMPLING INGREDIENTS, PRODUCTS, LIQUIDS AND RINSES

Our experience has shown the vast majority of errors associated with food testing can be attributed to how the original sample was obtained. Taking steps to ensure the sample to be tested is representative of the product as a whole will increase confidence in subsequent test results.

What follows are generally recommended guidelines for ingredient and product sampling, according to material type. Questions about the adaptability of the guidelines to specific testing needs can be directed to Neogen.

A. Dry, blended or finished ingredients and products

1. Obtain a 500 g sample from the ingredient or product to be tested and place in a clean container.
2. Thoroughly mix/blend the 500 g sample with a clean spatula or blender for at least 30 seconds.
3. Remove a 50 g subsample from the 500 g sample.
4. If the product has a large particle size, place the 50 g in a grinder and grind to a very fine particle size.
5. Thoroughly mix/blend the subsample with a spatula or blender for at least 30 seconds.
6. From the 50 g, remove 5 g for testing with one of Neogen's food allergen test kits. *Note: We recommend that the remainder of the sample be saved for confirmatory testing should a food allergen be detected.*
7. Thoroughly clean the grinder/blender and utensils between samples.

B. Liquids and clean-in-place (CIP) rinses

For homogenous liquids, it is not necessary to sample a large quantity. Simply draw 5 mL from the product or rinse to be tested for use with one of Neogen's food allergen test kits, and add to 125 mL of extraction solution. *Note: We recommend that at least 10 mL of the product or rinse be saved for confirmatory testing should a food allergen be detected.*

EXTRACTING ALLERGENS FROM INGREDIENTS, PRODUCTS, LIQUIDS AND RINSES

Neogen's peanut, almond, egg, hazelnut, soy flour, and total milk allergen kits utilize the same format for extracting samples using a heated shaker water bath. An alternative blending extraction is acceptable for peanut, almonds, eggs, hazelnut, and soy flour. Neogen's gliadin test kits utilize a separate format for extraction (see the next section if extracting gliadin). Instructions for each method follow. To extract food allergens residues from swabs, use the environmental swab extraction method described later in this handbook.

A. Water bath extraction method

Can be used with Neogen's quantitative and screening food allergen tests for almond, peanut, egg, hazelnut, soy flour, and total milk.

1. Prepare the extraction solution.
2. Obtain a representative sample. If the sample is of a larger particle size, grind it to a very fine particle size.
3. Transfer 5 g of sample, or 5 mL of liquid sample, to an extraction bottle.
4. Add one level scoop of extraction additive to the bottle.
5. Pour 125 mL of the extraction solution to the bottle, and cap the bottle.
6. Extract by shaking (150 rpm) in a water bath at 60°C (140°F) for 15 minutes. Remove the bottle from the bath.
7. Let material sit for 10 minutes to enable some of the sample to settle.
8. Use the clear supernatant as the sample for allergen testing. *Alternatives: Filter using a Neogen filter syringe or Whatman #4 filter, or centrifuge at 14,000 rpm for 5 minutes (20 minutes at lower speeds).*

B. Blender extraction method

Can be used with Neogen's screening tests for almond, peanut, egg, hazelnut, and soy flour.

1. Prepare the extraction solution and preheat to 60°C (140°F).
2. Obtain a representative sample. If the sample is of a larger particle size, grind it to a very fine particle size.
3. Transfer 5 g of sample, or 5 mL of liquid sample, to a 250 mL blender jar.
4. Add one level scoop of extraction additive to the blender jar.
5. Pour 125 mL of the 60°C (140°F) extraction solution into the jar and blend at high speed for 2 minutes.
6. Let material sit for 10 minutes to enable some of the sample to settle.
7. Use the clear supernatant as the sample for allergen testing. *Alternatives: Filter using a Neogen filter syringe or Whatman #4 filter, or centrifuge at 14,000 rpm for 5 minutes (20 minutes at lower speeds).*

EXTRACTING GLIADIN FROM INGREDIENTS, PRODUCTS, LIQUIDS AND RINSES

For analyzing heat-processed samples for the presence of gliadin using either Neogen's screening or quantitative format, follow extraction procedure C. For all commodities that were not heat-processed, follow either extraction procedure A or B. Samples of an unknown origin should be extracted using extraction procedure C.

Procedural note: If testing commodities where **dark chocolate, cocoa, and/or tannin** are the major ingredients (>90%), such as dark chocolate bars and cocoa powder, contact Neogen for a special extraction additive (Neogen item #8482). Add one scoop of this special extraction additive in addition to one scoop of the additive supplied with this test kit.

A. Extraction of non-heat-processed samples with orbital shaker or rotator

1. Prepare 40% ethanol extraction solution by combining 4 parts ethanol with 6 parts distilled water. Prepare sample extract dilution solution (PBS).
2. Add 1 g ground sample, or 1 mL liquid sample, to a clean 50 cc tube.
3. Add 1 scoop of extraction additive to the tube (see procedural note above).
4. Add 10 mL (9 mL for liquid samples) of 40% ethanol to the tube, cap tightly, then shake the tube vigorously by hand for about 20 seconds, or vortex for 10 seconds, to ensure complete mixing.
5. Extract by shaking (150 rpm) in an orbital shaker or rotator by laying down the tube on its side over the flat pad of the instrument, and holding it tightly using a rubber band or tape. Rotate or shake for 15 minutes at room temperature.
6. Remove the tube and let it stand in a rack for about 10 minutes to enable the sample extract to settle before withdrawing the clear extract.
7. Dilute each sample 1:40 by withdrawing 100 µL of the upper layer of the extract and transferring it to a small tube or vial containing 3.9 mL of PBS, or mix 0.5 mL of extract with 19.5 mL of PBS in a test tube.
8. To mix, vortex the tube for 5 seconds, or invert several times by hand.
9. Test diluted samples within 2–3 hours of extraction.

B. Extraction of non-heat-processed samples with shaker or shaker water bath

1. Prepare 40% ethanol extraction solution by combining 4 parts ethanol with 6 parts distilled water. Prepare sample extract dilution solution (PBS).
2. Add 2 g ground sample, or 2 mL liquid sample, to a 125 mL clean extraction bottle.
3. Add 1 scoop of extraction additive to the bottle (see procedural note above).
4. Add 20 mL (18 mL for liquid samples) of 40% ethanol, cap the bottle tightly, then shake vigorously by hand for about 20 seconds to ensure complete mixing.
5. Extract by shaking (150 rpm) in a shaker for 15 minutes at room temperature (a shaker water bath can work, but do not turn the heat on). Remove the bottle from shaker or bath.
6. Let the bottle stand for about 10 minutes to enable some of the sample to settle before withdrawing the clear extract.
7. Dilute each sample 1:40 by withdrawing 100 μ L of the upper layer of the extract and transferring it to a small tube or vial containing 3.9 mL of PBS, or mix 0.5 mL of extract with 19.5 mL of PBS.
8. To mix, vortex the tube for 5 seconds, or invert several times by hand.
9. Test diluted samples within 2–3 hours of extraction.

C. Extraction of heat-processed commodities

Heat-processed commodities require an extraction cocktail solution (Neogen item #8483) that re-natures the heated gliadin and allows the accurate detection of any possible gliadin in a sample. To extract gliadin from heat-processed samples:

1. Prepare 55% ethanol extraction solution by combining 55 parts ethanol with 45 parts distilled water.
2. Prepare sample extract dilution solution (PBS). *Note: Extraction with extraction cocktail solution should be performed under a chemical hood.*
3. Weigh out 0.25 g sample to 50 cc screw cap centrifuge tube.
4. Add 2.5 mL of extraction cocktail solution (dilution factor 1:10).
5. Cap and vortex 10-20 seconds to homogenize cocktail and sample.
6. Incubate 40 minutes at 50°C (water bath or oven).
7. Remove samples and let cool for 5-10 minutes.
8. Add one scoop of powdered additive (see procedural note above).
9. Add 7.5 mL of 55% ethanol and vortex again for 10-20 seconds (the final concentration of ethanol will be 41% and the sample dilution to this point is 1:40).
10. Shake (150-200 rpm) for 1 hour at room temperature on a rotator (tube on its side).
11. Centrifuge sample (if necessary) for 5 minutes at 2500 rpm.
12. Dilute the sample 1:10 into PBS (200 μ L sample into 1.8 mL PBS).
13. Samples are ready to run (1:400 final dilution).

ENVIRONMENTAL SAMPLING AND EXTRACTION

Environmental sampling for food allergen detection should be performed after equipment has been thoroughly cleaned, and before production of the following lot has begun. Because environmental sampling requires the use of swabs and a specialized extraction of possible allergens from the swabs, we recommend the use of Neogen's kit designed specifically for this purpose. The kit provides everything necessary to obtain an environmental sample for use with one of Neogen's food allergen test kits.

If you are not using Neogen's environmental sampling kit, swabs should be tested to ensure they do not cross-react with Neogen's food allergen test kits.

Whether or not the Neogen sampling kit is used, sampling should include areas known to be hard to clean in the environment to be tested. These may include equipment and conveyor nooks and crevices, scarred work surfaces, or any area where food residue buildup is a known concern.

We recommend the following procedure for environmental testing:

A. Prepare extraction solution

For testing for peanut, almond, egg, hazelnut, soy flour, and total milk

1. Prepare the extraction solution by adding the foil pouch of 10 mL PBS dry powder provided with Neogen's food allergen test kits to 1 L of distilled or deionized water at pH 7.4.
2. Preheat extraction solution to 60°C (140°F) by immersing the bottle containing the solution into a water bath and allowing it to reach 60°C.

For testing for gliadin

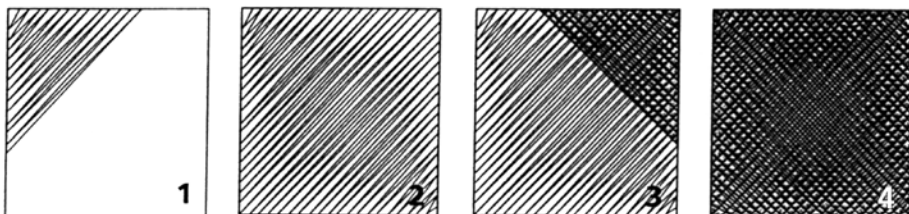
Note: If testing heat-processed samples for gliadin, refer to kit for additional instructions.

1. Prepare 40% ethanol solution by combining 4 parts ethanol with 6 parts distilled or deionized water (e.g., combine 200 mL ethanol with 300 mL water).

B. Swab and extract

1. Gather the sample with a swab, using one of the following methods:
 - a. *For dry surfaces:* Open a new swab and wet with extraction solution. Swab a 10 x 10 cm area by using the crosshatch technique.
 - b. *For wet surfaces:* Open a new swab and swab a 10 x 10 cm area by using the crosshatch technique. Do not moisten swab prior to use.

Note: When testing equipment, the swabbing area should be carefully chosen. Areas to swab should be potential food hangup areas, such as scarred surfaces, corners, angles, and any known food buildup area. An effective swabbing procedure, such as the crosshatch technique (illustrated below), should be used to ensure detection of any present allergen proteins by covering virtually 100% of the chosen area.



2. Return the swab to its original tube once sampling is complete. Remember to label each tube.
3. When ready to test, remove the swab from its tube, and add 5 mL of the appropriate extraction solution to the tube. PBS extraction solution needs to be warmed to 60°C before adding to the tube. (Do not heat ethanol solution.) Mix by placing the swab back into the tube and shaking for 2 minutes by hand (inverting tube), or for 30 seconds with a Vortex mixer.
4. Remove the swab from its tube, and place a new sample dropper tip onto the tube. (Gliadin extracts must be diluted prior to testing.) Remove the dropper tip from a new, prefilled dilution bottle. Add 3 drops of sample extract from the tube to a prefilled dilution bottle. Place the dropper tip back into the dilution bottle, swirl to mix.
5. The solution in the tube (dilution tube for gliadin) now serves as your sample for use with an Alert or Reveal allergen test kit. Continue sample analysis by following instructions specified in the allergen test kit.

Note: Use caution when inverting the tube, as some liquid may drip.

REVEAL FOR FOOD ALLERGEN TEST PROCEDURES

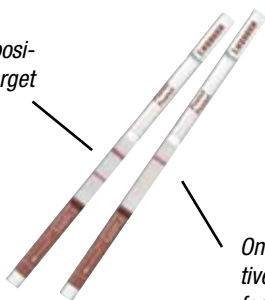
A. Reveal for Peanut Allergen

1. Transfer 0.5 mL of the sample extract to a sample tube.
2. Place a strip with the sample end down into each sample tube.
3. Allow each strip to develop in the sample tube. If two lines develop within 10 minutes, the sample is positive for peanut. If only one line develops, the sample is negative.

B. Reveal for Total Milk Allergen

1. Add 100 μ L of sample diluent to each microwell and swirl to mix for 30 seconds.
2. Add 100 μ L of sample extract to each microwell and mix by pipetting up and down 3 times.
3. Place a strip with the sample end down into each microwell.
4. Allow each strip to develop in the microwell. If two lines develop within 5 minutes, the sample is positive for the milk. If only one line develops, the sample is negative.

Two lines indicate the test is positive for the presence of the target food allergen.



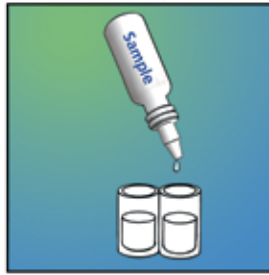
One line indicates the test is negative for the presence of the target food allergen.

SCREENING: THE ALERT FOR FOOD ALLERGEN TEST PROCEDURE

Note: Please read kit instructions completely before performing test.



1. Add 3 drops* (100 μ L) from the control dropper to the first well.



2. Add 3 drops of each sample extract to respective well. Mix. Incubate for 10 minutes.



3. Dump liquid from wells.



4. Wash wells thoroughly with washing solution. Tap out water on absorbent paper towel.



5. Add 3 drops* of conjugate to each well. Mix. Incubate for 10 minutes.



6. Repeat steps 3-4 by dumping out the liquid, thoroughly washing the wells and tapping dry.



7. Add 3 drops of substrate to each well. Mix. Incubate for 10 minutes.



8. Add 3 drops of Red Stop to each well. Mix.

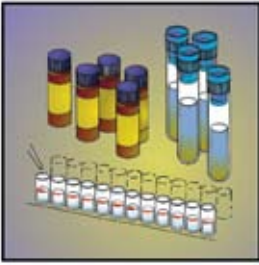


9. Visually read results.

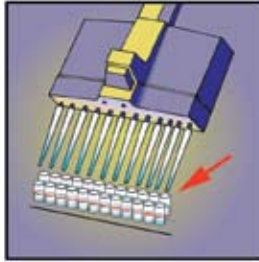
* Neogen requires the use of a pipettor instead of a dropper for these steps when testing for total milk to deliver precisely 100 μ L of liquid.

QUANTIFYING: THE VERATOX FOR FOOD ALLERGEN TEST PROCEDURE

Note: Please read kit instructions completely before performing test.



1. Add 150 μL controls and samples to transfer wells.



2. Transfer 100 μL to the antibody wells. Incubate for 10 minutes.



3. Dump liquid from antibody wells.



4. Wash wells thoroughly with washing solution.



5. Tap out water on absorbent paper towel.



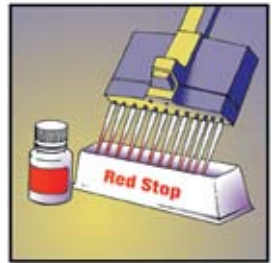
6. Transfer 100 μL conjugate to antibody wells. Incubate for 10 minutes.



7. Repeat steps 3-5 by dumping out the liquid, thoroughly washing the wells and tapping dry.



8. Transfer 100 μL substrate to antibody wells. Incubate for 10 minutes.



9. Transfer 100 μL Red Stop to antibody wells. Read results in a microwell reader.

HOW CAN ALLERGEN VERIFICATION FIT INTO A FOOD SAFETY PROGRAM?

Allergen verification can be included as a sanitation validation in a company's food safety program, or as part of a sanitation standard operating procedure (SSOP) when changing over from an allergen-containing product. Since allergens are classified as a chemical hazard by the FDA, with the unlabeled allergen the hazard, it is imperative to have an allergen control plan in place. Examples of possible strategies to control allergens in a food manufacturing facility:

A. Sanitation verification and validation using environmental swabs

1. Swab equipment surfaces prior to cleaning to assess the level of allergen present.
2. Clean production line according to the SSOP.
3. Swab equipment surfaces again to assess the level of allergen remaining.
4. Reclean if necessary.
5. Send non-allergen product through the equipment and test the first product produced. Test a representative number of samples throughout the first lot of product.
6. If the testing shows that the SSOP is adequate, then precautionary labeling should not be necessary.
7. All test product should be placed on hold or discarded.

B. Allergen verification as part of a food safety program

1. The production of safe food products requires that the food safety program be built upon a solid foundation, which may include, but are not limited to:
 - a. *Supplier controls.* Suppliers should be asked to provide information on their allergen prevention programs.
 - b. *Specifications.* All incoming ingredients should conform to specifications consistent with allergen control programs.
 - c. *Education.* All personnel should be trained about food allergen concerns.
 - d. *Product identification.* Product identification, traceability and recall procedures should be in place for all products produced.
 - e. *Good Manufacturing Practices (GMPs).* Examples of GMPs include:
 - Design of equipment for easy cleanup
 - Sanitation standard operating procedures (SSOPs)
 - Sanitation and control of receiving and storage areas
 - Sanitation and control of distribution points
 - f. *Identification of allergen sources.* Potential allergen sources include:
 - Raw materials
 - Ingredients
 - Sub-ingredients, e.g. natural flavors
 - Rework
 - Processing aids, e.g. wheat starch
 - Packaging materials
 - Cross-contact with shared equipment

2. Allergen verification can be quickly and easily performed using Neogen's screening and/or quantitative testing products. Specific areas that can be monitored include:
 - a. Incoming ingredients. Suppliers should have an allergen control program verified by first off testing to ensure their products are accurately labeled.
 - b. Environmental swabs. Swabs could be run after cleanup, and prior to the next production run. Any positive samples could indicate inadequate cleaning, and recleaning should be performed.
 - c. CIP solutions. A portion of the final CIP rinse solution can be tested. Any positive results may indicate inadequate cleaning, and additional steps may be required.
 - d. Hazard analysis. For initial identification of an allergen hazard during the risk assessment, a quantitative level of allergen is needed. Examples include assessing the undeclared allergen levels in push-through product, final product, etc.

EXAMPLE: ALLERGEN VERIFICATION IN A FOOD SAFETY PROGRAM

Company A, a bakery manufacturer, produces 3 flavors of cookies in the same facility: vanilla, sugar, and peanut butter crunch. As part of its allergen control plan, Company A asks its supplier to certify non-peanut ingredients are peanut-free. To further minimize the risk of peanut contamination, Company A follows the following system as a part of its overall food safety program:

A. Testing of raw material

Every 5th load of incoming raw material is tested for peanut allergen using the Alert for Peanut Allergen screening test, and delivery is not accepted until a negative test result is received. In the event of a positive sample, the delivery is either rejected or rerouted.

B. Testing of food contact surfaces

After the peanut butter crunch cookies are run, the processing equipment is cleaned. After the cleanup step, but prior to the next production run, each piece of equipment is swabbed, and swabs are screened for peanut residue. In the event of a positive result, the line is recleaned and retested.

C. Quantitative testing

In instances where a quantitative level of peanut is needed (e.g., to determine the effect of the ingredient in a final product), the Veratox test kit is used.

FOOD ALLERGEN SELF-EVALUATION CHECKLIST

A. Prior to initiation of a food allergen verification program

- 1. Is your staff, including part-time and temporary employees, trained on the severity of food allergies and the impact of recalls due to undeclared food allergens?
- 2. Is there a clear and defined labeling strategy, including:
 - a. Use of simple and everyday terms (e.g., “milk” instead of “calcium caseinate”)?
 - b. Is “may contain...” or similar labeling used only after thorough GMPs and SSOPs have been created and followed with respect to all allergens?
 - c. Is “may contain...” or similar labeling used only when the food allergen cross-contact is uncontrollable, sporadic and documented?
- 3. Have suppliers and co-packers been included in your food allergen control plan?
 - a. Have allergen questionnaires on ingredients been sent to all suppliers and received back?
 - b. Are there supplier/co-packer audits in place?
 - c. Are suppliers and co-packers aware of your food allergen control expectations?
- 4. Is there a clear and defined recall strategy in place?
- 5. Are there clear and defined consumer response strategies in place?

B. System design and product formulation

- 1. Is production equipment designed for easy, thorough cleaning, allowing the complete cleaning of filler heads, valves, belts and other equipment as necessary?
- 2. Have allergenic products been introduced into the production process at the latest possible stage of production?
- 3. Have allergenic products been scheduled at the end of a production shift, prior to clean-up?
- 4. If a non-allergenic product is following an allergen containing product in production, is a full allergen clean-up done?
 - a. Are all food contact surfaces “visibly clean” (no visible product remains)?
 - b. Is the “allergen clean” validated?
 - c. Is there a routine cleaning procedure verification program in place?
- 5. Have transportation vessels, including totes and pallets, been dedicated to allergenic product or rework?
- 6. Is there a “like into like” rework restriction?

C. Raw ingredients

- 1. Are any ingredients derived whole, or in part, from allergenic sources?
- 2. Is allergen information available from every supplier for every ingredient?
- 3. If the ingredient has been determined to be an allergen source, will another ingredient work equally as well?
- 4. If the ingredient has been determined to be an allergen source, does it have a functional affect on the product? If not, is it necessary?
- 5. Is each ingredient package or shipment of allergenic product clearly marked and identified as allergenic upon receipt?
- 6. Is there a clearly defined tracking strategy for each allergenic ingredient?
- 7. Is there a verification program to ensure ingredients are not a source of allergen?
- 8. Are allergenic ingredients stored in dedicated areas?

SOME RECOMMENDED TEST POINTS FOR VALIDATION OF ALLERGEN CONTROL STRATEGIES

A. Sanitation

1. All food contact surfaces which have come into contact with an allergen at some point.
2. Totes, pails, etc., used to transport allergens, if not dedicated.
3. All cleaning utensils used to clean production equipment where an allergen has been run, i.e., brushes, rags, scrubbers, dust collectors, etc., if not dedicated.
4. All sampling devices used to draw samples from an allergenic run.
5. Any push-through product used to clean-out prior to allergenic product.
6. All rework (if not from a like product).
7. Final CIP rinse.

B. Ingredients and raw materials

1. Any ingredient or sub-ingredient derived from an allergenic source (unless verified information from supplier indicates no issues), or those that may have come into contact with an allergen.
2. All natural and artificial flavorings, spices and additives which may be derived from an allergenic source, or have come into contact with an allergen (unless verified information from a supplier indicates no issues).
3. Any product not previously tested where a change in ingredient or formulation has been made.

C. Finished and in-process product

1. First product after changeover from allergenic to non-allergenic product (gluten to gluten free product).
2. Products where “may contain...” or similar labeling is used, to justify use of the statement.
3. Investigating food allergic consumer complaints about finished products.
4. All non-allergenic in-process product which has followed an allergenic run.

QUESTIONS AND ANSWERS REGARDING FOOD ALLERGEN TESTING

1. What is the difference between the terms “milk” and “total milk”?

“Milk” is a general term for a product that may or may not include both of the major dairy proteins—whey and casein. Since both casein and whey can be allergenic, Neogen uses the term “total milk” for its test to indicate that both casein and/or whey can be detected using the same test kit.

2. What is the relationship between “gliadin” and “gluten”?

Gluten is the major protein in wheat, rye, and barley. Gliadin is one of two prolamins of gluten that make up the gluten protein. The total protein content of gluten is approximately 50% gliadin. To determine the gluten content in a sample from a Neogen test for gliadin, simply multiply the sample’s test result by two.

3. Can a blender method be used for extraction?

Yes, a simple blender extraction method has been validated for use with Neogen’s screening tests (Alert and Reveal) for almond, peanut, egg, hazelnut, and soy flour. The blender extraction method does not yield acceptable results when used with Neogen’s screening tests for total milk or gliadin, or any of Neogen’s quantitative Veratox food allergen tests. All Veratox, with the exception of gliadin with its unique extraction protocol, allergen tests require the use of a shaker water bath method for sample extraction.

4. Can a handshaking method be used for extraction?

A handshaking extraction method can be used when extracting environmental swabs for any allergen, and for testing liquid samples for milk allergen using Neogen’s Reveal for Total Milk Test.

5. Can lab cleanliness affect sample results?

Laboratory conditions can affect test results. Neogen tests are designed for on-site testing; however, they are extremely sensitive and can unintentionally detect allergenic proteins that may exist in laboratory environment. Therefore, it is highly recommended that the sample preparation and testing areas, and all instruments, be regularly cleaned.

6. How long can sample extracts and swabs be stored before testing?

Swabs can be stored for up to 24 hours at 4°C after sample collection provided they have not been extracted yet. Once extracted, samples from swabs should be evaluated within 4 hours. All other sample extracts should also be tested within 4 hours.

7. Where are the best locations to sample the environment with swabs?

To yield test results that reflect true environmental conditions, samples should be taken not just from food contact surfaces, but also from corners, scarred work areas, screw heads, and any other areas where there is potential for food hang-up.

8. When should you test CIP rinse solutions?

In some closed systems where environmental sampling is not possible with a swab, clean-in-place (CIP) final rinses may be the only other option than product testing for verifying sanitation cleanliness.

9. Why is ATP testing not effective for food allergen monitoring?

ATP (adenosine triphosphate) is a substance in all organic matter, living or dead, and hence is not specific enough for allergen verification. No matter how sensitive the ATP claim, there is no way to differentiate ATP from an allergenic protein from that of all other sources of ATP. Also, many allergenic foods contain very low levels of measurable ATP, which would cause potential false negative results if testing for a food allergen using an ATP method.

10. How does heat processing affect the recovery of food allergens on the Neogen test kits?

Neogen has carefully designed its test kits to accommodate heat processed and highly processed samples. In some cases (e.g., its gliadin test), a special additive is used for processed samples. In rare cases highly refined proteins may not be detectable (see next question).

11. Are Neogen's allergen test kits appropriate for all samples?

Food allergens cannot be detected in some specific sample types by any commercially available test kit. Fermented and hydrolyzed proteins, as well as fermentation substrates such as gums, may not be detected due to the nature of the proteins, but there could still be active allergenic protein residue present. Food allergens may also not be detectable in some concentrated food additives, colors, and flavors. Contact Neogen if you have a question about a specific commodity.

12. What is the role of the “additive” in the extraction process?

An extraction additive plays two roles simultaneously—it enhances the solubility and stability of the allergenic protein, and eliminates background interference contributed by the food matrix being tested.

13. Are their analytical confirmatory methods for food allergen testing?

Currently, no confirmatory methods beyond ELISA exist. However, for confirmation of test results, there are many third party laboratories that run full, quantitative ELISA methods. While instrumental methods such as PCR and GC do exist, they are not recognized beyond the scope of research purposes only. Refer to the back of this handbook for a list of qualified labs.

14. Why are the Alert and Reveal test kits set at 5 ppm and 10 ppm?

Because regulators have not set actual thresholds for allergens, the food industry has taken a proactive approach of self governance and chosen these levels. They are relevant by minimizing risk to the consumer, without going to “zero tolerance” as a threshold.

15. What does the term “limit of detection” mean? Is it different than “limit of quantitation”?

A test kit’s limit of quantitation (LOQ) refers to the lowest point that its results are quantifiable. As a general rule, ELISA’s identify this as the first non-zero control in that kit, which is the 2.5 ppm control in Neogen’s test kits (5 ppm in gliadin). The limit of detection (LOD) is the lowest point at which a result can be considered above background noise. A LOD is determined as the mean of 10 evaluations of a known negative sample, plus two standard deviations. However, results greater than the LOD but less than the LOQ are analytically valid for qualitative purposes only.

16. Should a sample that tests below 5 ppm on an Alert kit be called “negative”?

No. As indicated above, the 5 ppm level has been established and generally recognized by the food industry as an indicator of risk associated with testing. However, levels below 5 ppm do not mean the sample is negative, as levels between the detection limit and 5 ppm may still be allergenic. More appropriate would be the phrase: “Below limit of detection (BLD)”.

APPENDIX A: THE PRODUCT SPECIFICATIONS OF NEOGEN’S VERATOX FOOD ALLERGEN TEST KITS

Product	Standards prepared from	Antibodies detect	Results reported as	Range of quantitation	Extraction
#8420 Veratox for Hazelnut	A mix of raw and roasted hazelnuts	Hazelnut proteins	Total hazelnut	2.5-25 ppm (100-1000 ng/mL)	1 to 25 in 10 mM PBS
#8430 Veratox for Peanut	22 varieties of raw and roasted peanuts	Peanut proteins	Total peanut	2.5-25 ppm (100-1000 ng/mL)	1 to 25 in 10 mM PBS
#8440 Veratox for Almond	A blend of common raw and roasted almonds	Almond proteins	Total almond	2.5-25 ppm (100-1000 ng/mL)	1 to 25 in 10 mM PBS
#8450 Veratox for Egg	Whole dried egg and baked whole dried egg	Unprocessed and heat-processed egg white proteins	Whole dried egg	2.5-25 ppm (100-1000 ng/mL)	1 to 25 in 10 mM PBS
#8470 Veratox for Total Milk	Nonfat dried milk	Caseins and whey proteins	Nonfat dried milk	2.5-25 ppm (100-1000 ng/mL)	1 to 25 in 10 mM PBS
#8480 Veratox for Gliadin	Wheat gliadin	Prolamins (wheat gliadin, rye secalin, and barley hordein	Gliadin (gliadin x 2 = gluten)	5-50 ppm (12.5-125 ng/mL)	*1:10 in 40% ethanol, then 1:40 in PBS (1:400)
#8490 Veratox for Soy Flour	Defatted, roasted soy flour	Soy flour protein	Soy flour	2.5-25 ppm (100-1000 ng/mL)	1 to 25 in 10 mM PBS

*Additional extraction procedures are required for dark chocolate, cocoa, tannin, and heat-processed samples.

APPENDIX B: THE TEST VALIDATION RESULTS FOR NEOGEN'S FOOD ALLERGEN TEST KITS

	Peanut	Egg	Milk	Almond	Gliadin	Soy flour	Hazelnut
Limit of determination (ppm)	2.5	2.5	2.5	2.5	5.0	2.5	2.5
Reproducibility: Intra-assay variability (%)	3.5	2.8	4.6	2.6	4.4	4.5	3.3
Inter-assay variability (%)	6.4	3.6	7.2	4.3	3.2	3.6	3.9
Linearity: r ²	>0.98	>0.98	>0.98	>0.98	>0.98	>0.98	>0.98
Cross-reactivity	No	No	No	No	No	No	*Yes
Recovery (mean %)	84	90	78	82	86	94	94
Stability (months)	>9	>6	>6	>6	>6	>6	>6
Assay time (min)	30	30	30	30	30	30	30

*<0.1% cross-reactivity to walnuts, <0.02 cross-reactivity to pecans.

RESOURCES

- **Neogen Corporation**, 517/372-9200; www.neogen.com (test kits, confidential allergen lab testing, allergen control verification programs)
- **FARRP**, 402/472-4430; www.farrp.org (food allergen consultation, allergen control strategies, confidential lab testing, training videos)
- **FDA**, www.cfsan.fda.gov/~dms/wh-alrgy.html
- **Food Allergy Issues Alliance Group**, www.nfpa-food.org (allergen labeling guidelines)
- **Food Allergy Anaphylaxis Network (FAAN)**, www.foodallergy.org (recalls, consumer complaints)
- **GMA/FPA**, www.fpa-food.org (food allergen consultation)
- **ABC Labs**, 352/372-0436, www.abcr.com (confidential lab testing)
- **American Institute of Baking (AIB)**, 800/633-5137, www.AIBonline.org (food allergen consultation, auditing, testing)
- **Health Canada**, www.hc-sc.gc.ca; 613/957-2991
- **Silliker Labs**, www.silliker.com; (food allergen consultation, auditing, testing)
- **Association for Dressings & Sauces**, www.dressing-sauces.org
- **Institute of Food Technologists**, www.IFT.org
- **Food Safety Net Services**, www.food-safetynet.com

Neogen Food Allergen Tests

- 8420 **Veratox for Hazelnut Allergen** - range 2.5-25 ppm, up to 38 samples.
- 8430 **Veratox for Peanut Allergen** - range 2.5-25 ppm, up to 38 samples.
- 8431 **Alert for Peanut Allergen** - qualitative at 5 ppm, up to 20 samples.
- 8438 **Reveal for Peanut Allergen** - qualitative at 5 ppm, 25 samples.
- 8440 **Veratox for Almond Allergen** - range 2.5-25 ppm, up to 38 samples.
- 8441 **Alert for Almond Allergen** - qualitative at 5 ppm, up to 20 samples.
- 8450 **Veratox for Egg Allergen** - range 2.5-25 ppm, up to 38 samples.
- 8451 **Alert for Egg Allergen** - qualitative at 5 ppm, up to 20 samples.
- 8470 **Veratox for Total Milk Allergen** - range 2.5-25 ppm, up to 38 samples.
- 8471 **Alert for Total Milk Allergen** - qualitative at 5 ppm or 10 ppm, up to 20 samples.
- 8478 **Reveal for Total Milk Allergen** - qualitative at 5 ppm, 24 samples.
- 8480 **Veratox for Gliadin** - range 5-50 ppm, up to 38 samples.
- 8481 **Alert for Gliadin** - qualitative at 10 ppm, up to 20 samples.
- 8490 **Veratox for Soy Flour** - range 2.5-25 ppm, up to 38 samples.
- 8491 **Alert for Soy Flour** - qualitative at 5 ppm or 10 ppm, up to 20 samples.



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