

A close-up photograph of a stack of chocolate bark. The top layer is white chocolate with green and orange sprinkles. Below it is a layer of dark chocolate with hazelnuts and green sprinkles. The bottom layer is dark chocolate with almonds and green sprinkles. The background is a wooden surface with more chocolate bark and a single almond.

Best Practices for Food Allergen Validation & Verification

► 1st Edition

Content

Food Production Allergen Validation and Verification of the Environment.....	3
Cleaning to a Validated Standard.....	3
Migrating from Validation to Verification.....	6
Multiple Allergens in Product.....	7
Selection of Test Method.....	7
Other Testing Considerations.....	7
Revalidation.....	9
Allergen Cleaning and Sanitation Documentation.....	9
Where to Test.....	10
Final Product Testing.....	10
What If I Can't Clean to the Target Level?.....	11
Should Allergen Advisory Statements on Ingredients be Carried Forward?.....	11
Resources.....	11

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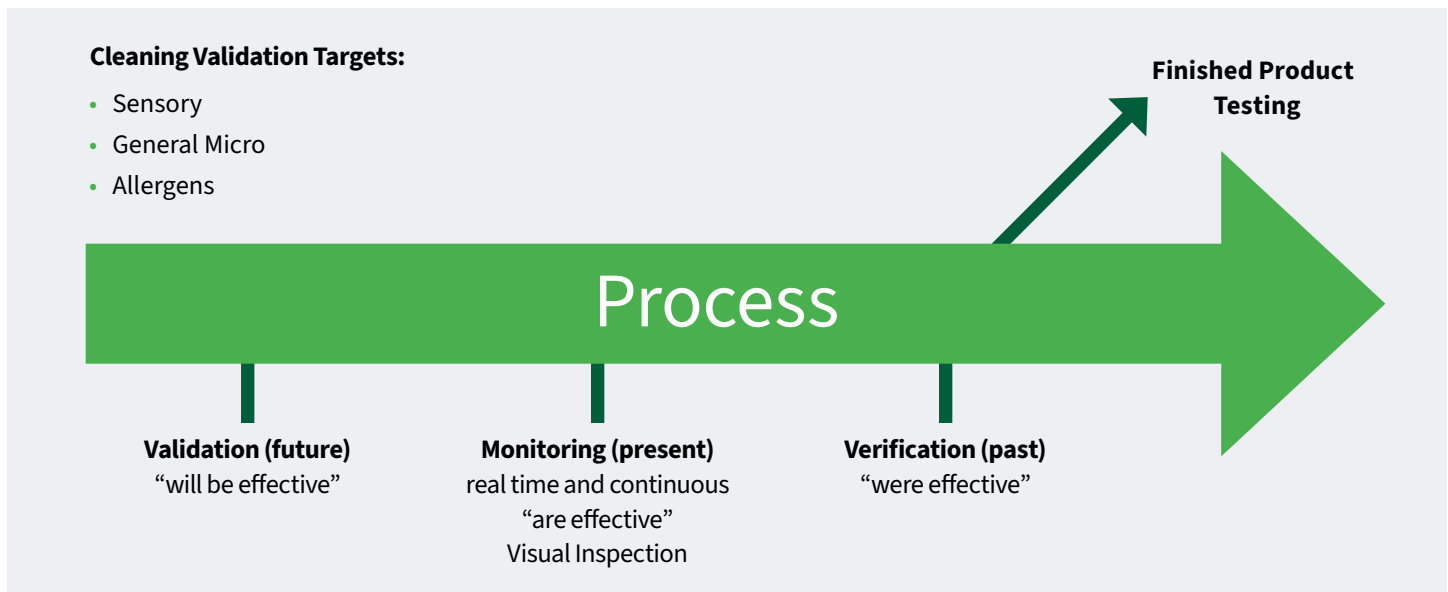
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Food Production Allergen Validation and Verification of the Environment

The definition of “validation” as given by Codex Alimentarius “GUIDELINES FOR THE VALIDATION OF FOOD SAFETY CONTROL MEASURES” (2008): “Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome.” In this case, the process refers to the facility’s materials and procedures utilized for cleaning after a production run containing allergenic ingredients. The underlying goal for the cleaning process in a food production environment is that it effectively removes all particulates, residues and microbial organisms to a safe and satisfactory level. Validation is proof that the goal can be achieved. It must be based on logical inferences and measurable results and those results must be translatable to standards that can be utilized for routine monitoring during the normal production cycle. Validation is typically undertaken until the expected outcomes are achieved and then repeated on a scheduled basis or when the underlying assumptions used for validation have changed.

Per Codex Alimentarius, verification is the application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended. This activity is typically undertaken after each cleaning event and results are compared against the performance levels obtained during the validation process. Results that fall outside the validated standard indicate that one or more components of the cleaning process failed. A facility’s verification process is typically incorporated into its Standard Sanitation Operating Procedure (SSOP).

Best practices for this document are defined as those that minimize risk of allergenic cross-contact between food production runs.



Cleaning to a Validated Standard

Cleaning in a food production environment is a critical base to any facility’s food safety program. The importance of an effective cleaning process can not be overstated. Failures can result in biological, chemical (including allergens) or physical material contamination of future production.

The challenge for most food production facilities is in establishing objectives and standards that can be measured in a meaningful way. Since most food contaminants that can represent a safety issue for consumers are either microbial or chemical/allergenic proteins, the optimal cleaning process will result when these entities are either removed and / or rendered inert. For that reason, most facilities utilize a cleaning process, which focuses on the removal of the soil that can house these contaminants, followed by a sanitation step to render any remaining microbial organisms inert.

Allergen cleaning validation is specific to each unique product produced by the facility. Therefore, it is necessary to perform a validation for each product risk profile. A possible exception would be if there were two different formulas but the ingredient differences were solely with non-allergenic flavorings, a validation would not be necessary for both products. Other exceptions may be applicable but must be justified. Once all cleaning processes have been established, a facility may choose to operate with separate SSOPs for the allergen containing product or standardize on the most rigorous SSOP across all product lines.

Test Kit Validation

It's important to note that any rapid food allergen method must be validated for the product being used. This is especially important as the trend in rapid food allergen test kits is for faster and simpler methods. The best way to determine fit-for-use is by running a positive control (allergen containing product) on each food type in your facility that contains the food allergen in question. The frequency for running the positive control is up to the individual facility but each food containing the allergen in question should read positive on the test. It is important to note that some lateral flow devices may be interpreted as negative on high positive samples. For that reason it is preferable to run a three line test with an "overload" line where available. If utilizing a two line test it may be necessary to perform a dilution until a positive result is achieved. The simplest way to do this is by spreading the food product on a surface, swabbing the surface and testing the swab. Any sample that does not elicit a positive result should not be considered validated on the test kit and your test kit supplier should be contacted for further evaluation. In addition some companies may choose to spike the non-allergen containing product with the allergen to ensure matrix effects will not cause erroneous results.

In addition the ability of the test kit to detect the allergen in question from actual food contact surfaces within the facility should be challenged by sampling areas prior to cleaning to show the presence of the residue and following cleaning to demonstrate their absence.

Best Practice for Validating the Cleaning Process

Allergen guidelines are typically expressed in parts per million (ppm). This measurement is appropriate when considering final product concentrations but does not easily translate to environmental surfaces. For this reason it is recommended to utilize a scoring scale system for environmental swab results tested with a quantitative test. Scoring on a scale of Green, Yellow and Red eliminates the confusion of interpreting a ppm result. With this scale a Green result provides a high level of confidence that your testing results meet expectations. Results with a score of Yellow can identify where additional cleaning is necessary but can also demonstrate progress toward the goal of Green scores. Note: This scoring system only applies to environmental swab testing and not finished product testing.

Due to the sensitivity of the quantitative ELISA assays, any result above the published limit of detection (LOD) should be considered positive and re-cleaning should be done. If using a qualitative lateral flow device (such as Neogen's Reveal 3-D & Allergen Protein tests), yellow and red will correlate to a "positive" and "high positive" score. It's important to note that various commercially-available test kits have different LOD's, hence it is not possible to correlate a quantitative result with a qualitative result down to LOD levels. The above chart is a practical method for interpreting results.

Score:	Green	Yellow	Red
Quantitative Result:	Below Limit of Detection	N/A	Above Limit of Detection
Screening Result:	Negative	Low Positive	High Positive

Utilizing a Quantitative Allergen Test (such as Neogen's Veratox Tests)

1. Produce the allergen-containing product.
2. Clean following the established SSOP.
3. Take a series of swabs at each of the identified test points (census method), focusing especially on harborage areas.
4. Perform the quantitative tests and compare the resulting score to the table above.
5. If any score falls outside of level Green, re-clean the respective area and perform additional tests until level Green status is achieved.
6. In some cases it is recommended to test the "first off" product to validate areas where the visual inspection or swab collection are unavailable.

7. Incorporate the new protocol into the next cleaning event for the particular product line.
8. Repeat steps one through five until ALL test sites have achieved level Green status for three consecutive cleanings.
9. Document your process and the test results to support it. Make appropriate changes to the SSOP.

Utilizing a Screening Allergen Test (such as Neogen's Reveal 3-D & Allergen Protein tests):

When testing with a screening method, score a negative result as Green and a positive result as Yellow. In the event of an overload result (high positive) a Red may be scored.

1. Produce the allergen containing product.
2. Clean following the established SSOP.
3. Utilizing the screening tests' swabs, take a series of samples at each of the identified test points, focusing especially on harborage areas. Only the environmental swabs supplied or recommended by the test kit supplier should be used for allergen analysis.
4. Complete the screening tests noting the results as "Green" negative or "Yellow/Red" positive/high positive for the allergen of concern.
5. If any test site is positive, reclean the respective area and perform additional tests until a negative result is achieved.
6. Incorporate the new protocol into the next cleaning event for the particular product line.
7. Repeat Steps 1 through 5 until ALL test sites have achieved negative status for three consecutive cleanings.
8. Document your process and the test results to support it. Make appropriate changes to the SSOP.

Validation:

Test identified sites until each site achieves a Green score. Yellow scores can help in demonstrating progress toward the goal of Green scores for each site.

Sites	Date and Time Tested	Score Green, Yellow, Red	Retest Score G,Y,R	Retest Score G,Y,R

Determining Appropriate Testing Sites:

The objective for any environmental monitoring program is to achieve a representative sampling of the area of concern. While there is no right or wrong number of test sites, the focus should be on testing each site that represents a unique surface (material, complexity, location), unique cleaning protocol and unique product composition as well as those areas that represent unique cleaning challenges such as welds, corners and other harborage areas. Representative sampling for validation should also occur before and after thermal processes to ensure that the test can detect the allergen in raw and finished product.

A best practice approach to determining appropriate testing locations would include a consultation with sanitation and maintenance staff as well as equipment and sanitation chemical suppliers.

Typically, one sample per unique test site is appropriate although it is important to remember that a failed test will normally imply the re-cleaning and re-testing of the area and equipment represented by that test site.

Migrating from Validation to Verification

Once the cleaning validation has been completed successfully, a facility will typically evolve their monitoring program to a more routine check of adherence to the validated cleaning protocol. This verification is typically performed after each production run and associated cleaning event and is designed to reflect whether the cleaning process was completed successfully relative to that standard.

Since the sampling and methods utilized during the validation process may be too cumbersome to perform on this routine basis, most facilities operate with a verification program that features representative sampling with a combination of target allergen testing and a surrogate method such as ATP, protein or visual examination.

In order to know how best to interpret the results from a test such as ATP or protein testing, it is imperative to incorporate that testing into the final stages of the validation process. Once successful validation has occurred and an SSOP has been established, a facility should conduct a census of test sites.

Determining the Quantity of Tests Necessary for Verification

Consistent with the approach outlined in the “Determining appropriate test sites” section, the objective with routine monitoring and verification is to achieve a representative sampling for each cleaning event. The appropriate number of samples will be process and facility specific and must be considered within real-world production and budget requirements. The number may also evolve over time as the facility becomes more or less consistent with its cleaning.

A robust monitoring program identifies areas for additional consideration and can spot issues before they become problems. As an example, a facility may adopt a standard sampling protocol of five tests following each production and cleaning run for a particular product. If the data indicate a particular site is becoming more difficult to clean effectively and consistently, increased sampling of that area may be warranted. Numbers can also fluctuate due to the need for additional monitoring following cleaning staff turnover.

A robust monitoring program will take into consideration the number of potential test sites and the time it will take to sample each test site at least once. If, as an example, a facility chooses to test five sites each day at random out of a potential 50 sites, it will take ten days before each site can be reasonably assumed to have been tested. The same number of sites each day from a pool of 100 test sites would result in each site being sampled once each 20 days, or in a 20-day work month, once each month.

The most important consideration when determining the number of tests to perform following each cleaning event is that it is based on a solid foundation of logical and supportable thought. Auditors and stakeholders will typically concern themselves more with the logic behind the program than the details of the program.

Best Practice for Verification of Cleaning in an Allergen-Containing Production Environment:

Clean following a validated SSOP and use allergen-specific (ELISA) tests. Production can resume only upon negative test results. Some companies may choose to verify using another non-allergen specific test, such as ATP, general protein and visual observation. Note that these methods may not correlate to allergen-specific ELISA tests, and will not be indicative for the presence of allergens. If a surrogate method is used, it is recommended to generate side-by-side data for a period of time to determine correlation between methods.

Pre-Op vs Post-Op

There are two primary uses for the verification data. Each implies a different interpretation of the test results. In a post-operation (post-op) cleaning environment, the verification indicates that the previously produced material and any potential cross-contact residues have been removed from the production surfaces. In a pre-operation (pre-op) environment, the test results are utilized as an indication that it is safe to begin the next production run. In many production facilities, the test results can be used for both, depending on the length of time between production runs and the environmental conditions surrounding the production equipment. In general, the longer the time between production runs and the more activity around the equipment while it is idle, the more likely post-verification cross-contact has occurred. As an example, if a production line sits idle after cleaning for eight

hours and is near another production line producing a peanut and flour product, it's easy to imagine that particulates from that production could spread to the cleaned production line. In this example, the facility may:

1. Shift their cleaning and verification programs from immediately after the production run to a time period closer to the next production run,
2. Choose to segregate the equipment to avoid cross-contact,
3. Schedule a second, lighter version of their cleaning SSOP and verification pre-op, or
4. Cover open or exposed food contact surfaces with plastic sheeting/or other materials to protect against particulate and dust collection, or if possible some companies may remove certain pieces of equipment while not in use.

Multiple Allergens in Product

In cases where multiple allergens are present, it must be determined if these ingredients are potential risks for particulate cross-contact or are fully integrated into the formulation. If fully integrated, one can choose the allergen present in the greatest concentration or is perceived to be the most difficult to clean. An example of a difficult to clean allergen may be a peanut butter component that is particularly sticky versus a milk powder component which could be removed through normal cleaning procedures. An example of a particulate risk would be a chopped almond topping that could be harbored somewhere in the processing equipment and could shed intermittently onto subsequent batches of products. Particulate risk materials should be screened separately and individually. Where risk materials may be present in particulate form, visual inspection is a critical first screen. It may be appropriate to perform flush testing or “first product off the line” testing.

If particulate risk is low, choosing the most difficult to clean or most abundant allergen in the formulation is a suitable means of gauging the absence of the others.

In situations where no test kit is available to screen for a particular allergen, visibly clean remains a first standard in sanitation and can be verified with a surrogate system.

Selection of Test Method

Any analytical method selected for verification should be validated on-site in the user's facility, under its specific conditions, to assure the method will detect allergens of concern. This is known as testing a positive control.

- Allergen specific immunodiagnostic tests are highly recommended and considered the industry best practice for allergen cleaning validation.
- Surrogate methods, such as ATP and general protein swabs, may be considered acceptable for verification when:
 - specific immunodiagnostic test methods are unavailable for a particular allergen, or
 - the method has been validated in-house against the specific allergen.
- “Visibly clean” may be considered acceptable for verification, however, it needs to be consistent and validated against analytical, allergen specific (ELISA or lateral flow) testing.
- In instances where multiple allergens exist, it may be acceptable to choose one allergen to demonstrate the effective removal of all allergens, provided the worst case scenario (i.e., allergen in highest concentration or most difficult to clean) is chosen.

Other Testing Considerations

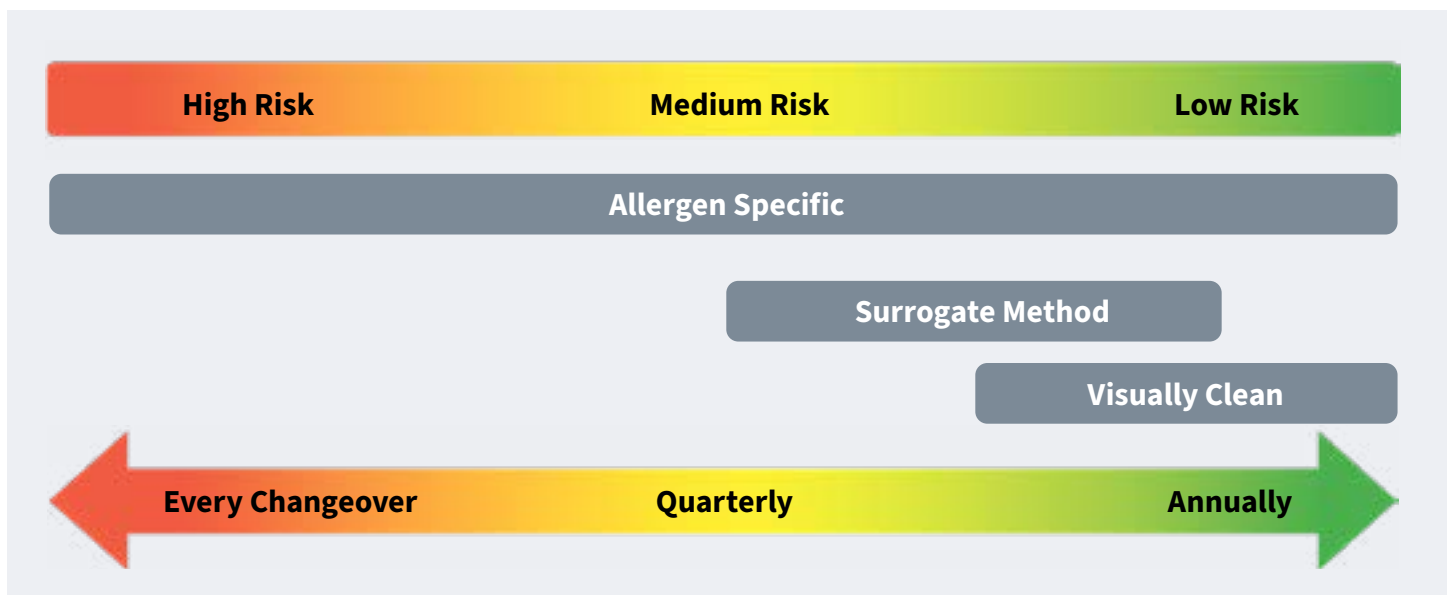
The following are suggested guidelines:

- Determine the risk incurred from raw material suppliers by evaluating ingredients for presence of an allergen to verify the supplier's Certificate of Analysis (COA).
- Using the guidelines above, select the allergens to screen. Using an antibody-based immunoassay suitable for environmental monitoring, evaluate the visibly dirty surface prior to cleaning to establish a baseline where positives can be obtained in this worst case scenario.

- Identify food harborage areas within the production line to focus specific attention.
- Devise a cleaning strategy consulting with sanitarians and cleaning chemical suppliers suitable for removing the soil.
- After the equipment has been suitably cleaned and passes all visible standards of clean, sample along the entire production line ensuring focus on the identified harborage zones.
- The presence of cleaners and sanitizers can affect limit of detection of test kits.
- This process should immediately follow every allergen production run and should be repeated three consecutive times without failure to validate the allergen cleaning process. Validation should reoccur at minimum annually and when those situations identified in the “Revalidation” section occur.

How often one should verify that there has been no drift in sanitation practices is another key question and cannot be answered until the level of tolerable risk can be established.

- **Highest risk:** When the product has the highest risk for inadvertent allergen cross-contact the most conservative approach would be to monitor environmentally upon changeover of every allergen production run to verify the existing sanitation protocol is still effective and document that verification using an allergen-specific immunoassay.
- **High-Medium risk:** Quarterly monitoring upon changeover with an allergen-specific immunoassay represents a less conservative approach.
- **Medium-Low risk:** Using a general protein or ATP system to monitor product changeovers for allergen removal represents a greater level of risk due to the potential for allergens to be present below the level of detection of general protein tests and the unknown or variable correlation between ATP and allergenic protein presence.
- **Lowest risk:** Perform environmental monitoring through visual inspection but there is an increased level of risk to the subjectivity of the observer, lighting conditions and other variables.



When a product has a “free from” claim, it is strongly recommended that in-process and finished product be tested to verify the claim. Given that these claims invite the allergic consumer to safely consume the product, they have the most risk associated and the most conservative approach to manage that risk should be taken. Therefore, the use of a quantitative test on finished product is essential.

Revalidation

Validation should be undertaken at least once each year unless a specific event has occurred to call into question the validated procedure. Events that would typically trigger the need to revalidate prior to the annual schedule

- Changes in raw material suppliers
- Changes in equipment layout or design
- Changes in production formulation, or introducing new or seasonal products
- Changes in chemical supplier or sanitation service provider
- Changes in packaging material – as an example, the incorporation of wheat starch to prevent cardboard from adhering during processing.
- Changes in allergen test kit or vendor

Allergen Cleaning and Sanitation Documentation

Once a validated allergen cleaning program has been established, and a verification schedule has been devised, it's important to document all elements of the program. As part of FSMA, the FDA has access to all Preventative Control documents, including allergen control, and may request them upon inspection. Below is a partial list of elements that should be documented in an allergen sanitation preventative control document:

1. The responsibility and methods used to control allergens and prevent cross-contact with dissimilar allergens or non-allergenic ingredients
2. A risk analysis of those ingredients, raw materials and processing aids that are used in the facility and are a potential risk for cross-contact
3. A register of all allergens within the facility pertinent to the country of manufacture, destination and labeling.
4. All hazards associated with allergens, and the corresponding preventative control. Controls may include, but are not limited to:
 - a. Ingredient specifications
 - b. Receipt and storage of allergenic ingredients
 - c. Production scheduling
 - d. Rework policy (if used)
 - e. Equipment design
 - f. Sanitation
 - g. Testing practices
5. Instructions on identification and segregation of allergenic materials
6. Cleaning and sanitation practices between allergenic and dissimilar or non-allergenic production runs, including validation and verification
7. Corrective action plans denoting actions and responsibilities in the event of a deviation

Where to Test

Sampling areas for Environmental Monitoring Programs (EMP) may be broken down into zones based on their proximity to the product. Zone 1 would typically be defined as surfaces that come into contact with the product. Zones 2, 3 and 4 would be non-contact surfaces of lessening probability that could contribute contaminants through some interaction with people, equipment or air and water circulation.

Zoning allows for monitoring to be conducted in environmental areas where the product and food contact surfaces are more susceptible to allergen cross-contact, to those areas that are far removed from the product, but may still have an impact on quality.

Zone testing can help to identify areas in the plant environment that may be contributing to allergen cross-contact of the product or food contact surfaces. Through data trending and result interpretation zone testing allows the plant to identify environmental areas or traffic patterns that can be a source of allergen cross-contact.

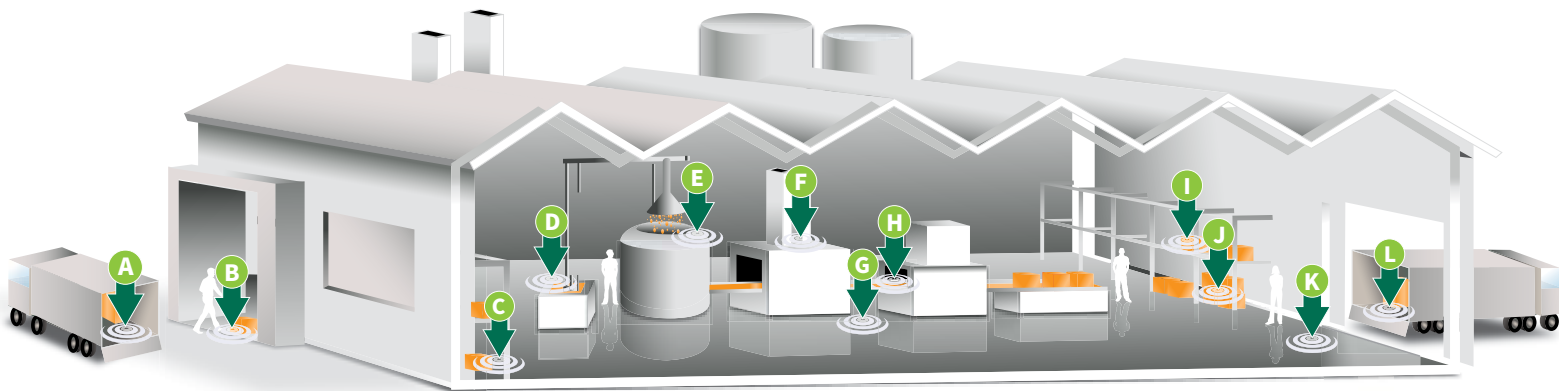
For allergen cleaning verification, testing sites should be concentrated in Zone 1 as this is where the product is most susceptible to allergen cross-contact. Testing these surfaces/areas will help to identify hot spots of allergen cross-contact that can directly affect the product and product contact surfaces. Testing in Zones 2, 3 and 4 can pinpoint allergen cross-contact sites outside of the production area.

By identifying allergen cross-contact sites, facilities can minimize contamination that otherwise would be brought into the production room(s) through employee and equipment traffic.

The number of swabs taken is unique to the plant and should be related to the level of risk identified in the initial risk assessment as well as other factors including the company's tolerance of risk, customer base, equipment design, age of equipment and ability to clean. In general however, more swabs should be taken in the earlier stages of production, and test sites should be focused on difficult to clean areas such as angles, welds, porous surfaces such as cloth belts and other harborage areas. Representative samples should be taken from dissimilar materials such as Teflon, belting and stainless steel.

Final Product Testing

Final product testing for allergens can be an important validation and verification tool for your overall food safety program. If during the risk analysis you've identified unintended food allergens as a risk, preventative controls should be in place to ensure the risk is eliminated. In this case, final product testing is encouraged. However, for the purposes of this document, final product testing should not be considered a validation or verification test, as issues such as sampling, random distribution, and product dilution may render test results meaningless. For cleaning validation and verification, only those tests that directly measure the effectiveness of cleaning should be performed.



Incoming Foods & Warehouse

A - AUDITING SUPPLY CHAIN

Verify allergen controls

B - INCOMING GOODS

Confirm supplier specifications

C - WAREHOUSE

Ensure robust ingredient segregation and packaging integrity

Processing & Packaging

D - FOOD PREPARATION SURFACE

Validate cleaning and detect cross-contamination

E, F - PROCESS EQUIPMENT AND UTENSILS

Validate cleaning and detect cross-contamination

G - SPILLAGES

Ensure verification of cleaning

H - IN-PROCESS AND RE-WORK

Detect potential cross-contamination

Finished Product & Warehouse

H - FINISHED PRODUCT

Verify product labelling claims

J - WAREHOUSE

Ensure robust final product segregation and packaging integrity

K - SPILLAGES

Ensure verification of cleaning and refer to control program for non-conforming product

L - FINISHED PRODUCT SUPPLY CHAIN

Auditing and enforcement

What if I Can't Clean to the Target Level?

There may be instances where all elements of the allergen control plan are followed, and you still cannot guarantee against cross-contact. In these cases precautionary ("may contain...") labeling may be the best course of action. However, to justify the use of precautionary labeling, each instance should meet the following four criteria:

1. Allergen must be documented as in the food environment, and is a risk for inclusion in product not intended to include the allergen
2. The risk or presence of the allergen is uncontrollable and cannot be minimized without major revisions to the manufacturing process or GMPs (note that FDA has stated that precautionary statements must be truthful and cannot be used in lieu of GMPs)
3. The food allergen is likely to be present in some, but not all, product where its presence is unintended
4. Consuming the allergen in a product where its presence is unintended would constitute a health hazard to a consumer allergic to the allergen

Source: *Managing Food Allergens in Food Processing Establishments; 4th edition 2009, Grocery Manufacturers Association*

Should Allergen Advisory Statements on Ingredients be Carried Forward?

It is important to first determine the circumstances leading to an ingredient utilizing an Allergen Advisory Statement. If it is determined that the above criteria are not met an effort should be made to work with the supplier to reconsider the use of allergen advisory statements. However, if it is determined that an Allergen Advisory Statement is required for the ingredient then typically an Allergen Advisory Statement should be carried forward to the label of the finished product. However, you may choose to exercise discretion based on inclusion rate, protein load or other supportable logic.

Resources

Neogen Corporation, 517/372-9200; www.neogen.com (test kits, confidential allergen lab testing)

FARRP, 402/472-4484; www.farrp.org (food allergen consultation, allergen control strategies, confidential lab testing, training videos)

Grocery Manufacturers Association (GMA), www.gmaonline.org



We are here to support you

Neogen Offers Global Support for Allergen Detection



Method Trial

- Technical sales support
- Education materials (by segment, competitive tech, assay type)
- Demonstration or initial evaluation



Method Application

- Matrix Qualification Program*
- Studies and publications
- Integration into lab workflow
- Training



Routine Use

- Product availability
- Results interpretation
- Technical concerns

* Contact your local Neogen representative for more information.

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