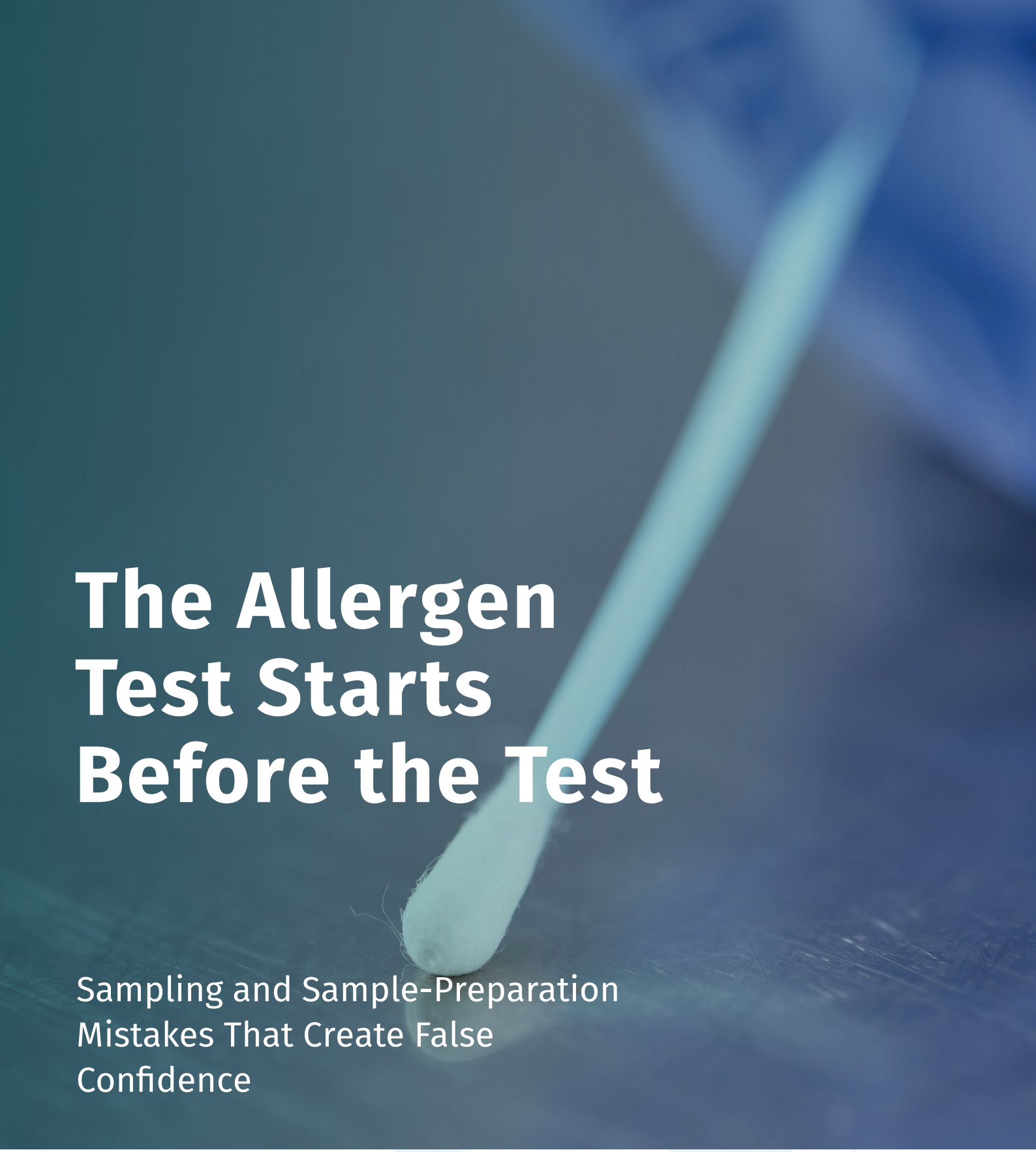

The Allergen Test Starts Before the Test

Sampling and Sample-Preparation Mistakes That Create False Confidence

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Making the World's Food Safer

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Executive Summary

Even when an allergen assay performs exactly as intended, results can still mislead if **sampling and sample preparation** do not reflect how allergen residues behave in real production environments. Allergen residues are often **heterogeneously distributed ("hotspots")**.

In dry handling they may be particulate (e.g., powders); in wet systems they may persist as **smears/films, dried residues, or partially soluble proteins.** As a result, location, timing, site definition, technique, handling, and method compatibility are decisive.

This whitepaper provides a practical approach to:

- facility-specific allergen risk mapping
- sampling plan design that clearly separates cleaning validation from routine verification
- sample-type-appropriate handling and extraction considerations
- common pre-analytical failure modes with corrective actions.

The objective is not a higher volume of testing - it is **risk-based testing** that produces defensible, actionable results for **line release, trend analysis,** and **incident response.**

Scope and intended audience

This document is intended for QA/QC, sanitation, operations, and food safety leaders designing or reviewing allergen control monitoring in food production environments. It focuses on **pre-analytical controls** - sampling location, timing, technique, handling, and sample preparation - for rapid immunoassays (LFD and ELISA), primarily in the context of **cleaning validation** and **routine cleaning verification.**

It does not replace regulatory or customer requirements, nor does it provide legal advice.

Key definitions

(used consistently throughout)

CROSS-CONTACT

Unintended presence of an allergen in a product due to transfer from allergen-containing materials, equipment, people, or the environment.

VALIDATION

Evidence that a cleaning/control measure can achieve intended allergen removal/reduction under defined (often worst-case) conditions.

VERIFICATION

Evidence that the validated cleaning/control measure was executed effectively for a specific event/day/run (e.g., pre-op line release after sanitation).

HOTSPOT / HARBORAGE POINT

A location where residues can persist due to design, wear, or cleaning limitations (e.g., seals, gaskets, dead legs/low-flow zones, worn belts, cracked plastics, rough welds, valves/nozzles).

UNIQUE TEST SITE

A distinct sampling location defined by surface material + geometry/complexity + cleaning method + product/allergen exposure + transfer risk (not merely “another nearby spot”).

ZONES (ALLERGEN ENVIRONMENTAL ZONING)

- **Zone 1:** direct food-contact surfaces
- **Zone 2**: adjacent surfaces with plausible transfer to Zone 1 (frameworks, guards, exterior of hoppers, equipment legs)
- **Zone 3**: within-room non-contact (floors, drains, carts, forklifts, door handles)
- **Zone 4**: remote/non-production areas (hallways, offices)

Note: zoning helps justify why some non-food-contact sites matter - especially **in dry/powder environments.**

1. The test can be perfect and still be wrong

Most facilities have experienced some version of this: sanitation is completed, verification swabs come back negative, and production moves on. The result looks clean. The decision feels safe.

Then reality checks back in - sometimes as a customer complaint, sometimes as a targeted investigation swab, sometimes as a second sample from a different location that tells a very different story.

When these mismatches happen, the assay is rarely the primary failure point. More often, the program lost signal earlier in the chain: **where the sample was taken, when it was taken, how it was handled, and what the method could realistically extract and detect** from that material.

This is the central premise of the paper: **the allergen test starts before the test.** If sampling and sample preparation don't reflect real transfer routes and real facility constraints, even a technically flawless assay can create false confidence.

2. Why sampling matters in allergen verification

Allergen risk behaves differently from many other hazards. Cross-contact is often:

Sporadic and intermittent: triggered by a specific changeover, shift practice, ingredient tote, maintenance event, or cleaning variation.

Rarely uniform: residues concentrate in hotspots rather than distributing evenly across a line.

Multimodal in transfer routes: people, dust, tools, traffic patterns, air movement, and hard-to-clean harborage points can move residues in ways routine sampling can miss.

That's why a negative result can be falsely reassuring. Sometimes the sampling plan missed the relevant location or time window and tested a low-risk micro-area while the true risk sat elsewhere. And sometimes the location and timing were appropriate, but **handling, extraction limits, matrix effects, or chemical residues** prevented detection.

The consequences are real: consumer harm, product holds, relabeling, rework, costly recalls, and lasting reputational damage. The good news is that many failures are preventable - with a sampling plan that matches how allergens persist and transfer in real facilities.

3. Step 1 - Identify where allergen risk exists in your facility

There is no universal sampling rulebook that covers every facility type, line layout, and product mix. Allergen risk is highly site-specific. A defensible program begins with **facility-specific risk mapping** grounded in the hazard analysis.

Start by mapping the process and identifying plausible cross-contact routes. Typical high-risk points include:

- **Shared equipment and changeovers** (especially where allergen-containing runs are followed by allergen-free runs)
- **Open handling areas** where dust or particles can travel (powder tipping, ingredient additions, open conveyors, bag dumps)
- **Communication between different areas**: air conditioning tubes, ventilation…
- **Design traps / hard-to-clean features** (gaskets, joints, dead legs, rough welds, worn belts, damaged seals, valves/nozzles, static seals, threaded fittings)
- **Rework loops and return systems** that blur product boundaries
- **People and traffic patterns** (gloves, aprons, shared utensils, forklifts, maintenance tools moving between zones)

If a location cannot be justified via the hazard analysis (credible transfer route + plausible persistence + plausible transfer into product), it is likely being sampled for convenience rather than risk control.

The goal isn't a perfect map. It is a useful one: a short list of sites where allergen residues are most likely to persist and most likely to transfer into product.

4. Step 2 - Build a HACCP - aligned sampling plan (and separate validation from verification)

HACCP is fundamentally about identifying hazards, implementing controls, and verifying that controls work. Allergens fit naturally into that framework, and auditors expect a clear distinction between **validation** and **routine verification**.

Validation vs. Verification (not interchangeable)

Validation (does it work under defined conditions?)
A validation study demonstrates that a cleaning method and SOP can remove allergen residues under defined (often worst-case) conditions. Validation is typically more intensive: more sites, replicates, multiple runs, and sometimes multiple approaches (e.g., allergen-specific swabs plus rinse testing where appropriate).

Routine verification (did it work this time?)
Routine verification demonstrates that the validated method was executed effectively for a specific event/day/run. Verification is targeted and consistent: a defined set of unique test sites used for pre-op checks or line-release decisions.

Blurring validation and verification undermines audit defensibility. Your sampling plan should explicitly label which activities are validation studies versus routine verification - and why.

The four questions every practical allergen sampling plan must answer

1) What decision are we supporting?

Typical decisions include:

- incoming material control
- cleaning verification between lots
- line release for allergen-free production
- root-cause investigation after a positive

2) Where should we sample?

Focus on **unique test sites** at worst-case points:

- harborage points (seals, gaskets, worn belts, cracked plastics)
- transfer points (where product passes into or through Zone 1)
- open handling zones (dust/particulate exposure)
- adjacent Zone 2 points that plausibly transfer to Zone 1

3) When should we sample?

Timing must match the decision:

- **pre-op (post-clean, immediately before start-up)** for line-release decisions
- **post-op (after production, before cleaning)** to identify accumulation points and target validation improvements
- **final product (just before packaging)** for lot release decision
- **after defined cleaning events** as required vby the verification program

Also consider **idle-time recontamination** risk (e.g., dust settling, traffic, maintenance). If that risk exists: verify closer to start-up, protect equipment, or strengthen procedural controls.

4) How many samples do we need?

The goal is representative, risk-based sampling.

- In **routine verification**, the practical unit is often **one sample per rigorously defined unique test site**, but only if swabbed area and technique are standardized.
- Where areas are large, residues are particulate, or harborage is complex, increase confidence by improving **within-site coverage** (defined pattern, defined area) or by splitting into **sub-sites** treated as separate unique test sites.

Minimum elements of a defensible cleaning validation study (often missing)

A validation plan should explicitly define:

- **Worst-case scenario(s)**: allergen type, soil load, run length, dry time/bake-on, equipment condition (worn parts), and hardest-to-clean formulation (e.g., high fat/sticky).
- **Sampling intensity:** more sites and replicates than routine verification; include disassembly points where feasible.
- **Acceptance criteria:** define “pass/fail” in advance (commonly **no detectable allergen** at defined critical sites using a validated method; or a documented quantitative criterion where appropriate).
- **Revalidation triggers:** changes to product/formulation, allergen profile, equipment design/condition, sanitation SOP, cleaners/sanitizers, CIP parameters, or line layout/airflow.

5. What to sample - four sample types with different representativeness risks

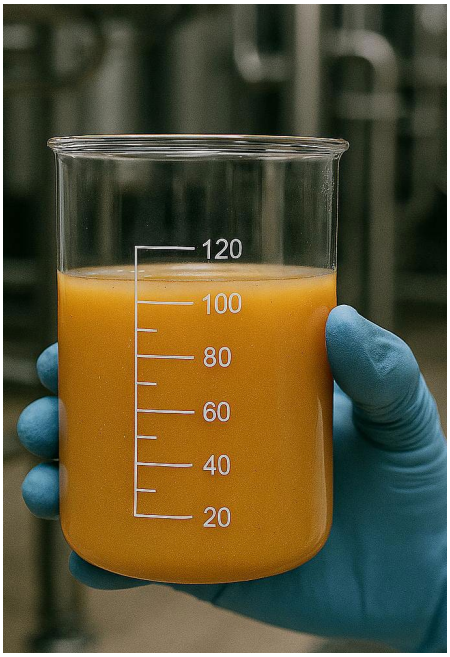
To keep an allergen program practical, it helps to think in four categories. Each behaves differently and requires handling aligned with validated test instructions.

5.1 Solid samples (ingredients, powders, intermediates, finished products)

Solids are often heterogeneous; allergens can clump or localize. The main risks are poor representativeness and incomplete homogenization. A grab from the top of a container may not represent the lot.

Practical controls

- use incremental or composite sampling where appropriate
- homogenize thoroughly (validated approach) before sub-sampling
- document sampling points and increments (especially for investigations)



5.2 Liquid samples (beverages, sauces, slurries)

Liquids can appear uniform, but viscosity, fat content, and phase separation can create pockets.

Practical controls

- standardized mixing and subsampling technique
- validate matrix suitability (fat, viscosity, pH can matter)
- avoid sampling after long hold times without re-mixing

5.3 Surface samples (swabs)

Swabs are central to cleaning verification and environmental control. Done well, they detect residue at meaningful transfer points. Done poorly, they can miss hotspots or introduce contamination via technique.

Practical controls

- standardize area, pattern, and pressure
- sample worst-case surfaces/materials and worn zones
- use the buffer and swab system specified/validated for the method

5.4 Rinse water / CIP return

For vessels and closed systems that are hard to swab, rinse water can be informative - provided detergents, sanitizers, temperature, and dilution effects on recovery and assay compatibility are understood.

Practical controls

- define which phase is sampled (often final rinse/return)
- understand dilution: “non-detect” can reflect dilution, not absence
- confirm compatibility with cleaning chemistry and sanitizer residues

Practical note: Methods are not interchangeable.

Sampling devices, buffers, and extraction procedures must be selected and used as validated for the intended sample type and matrix. Reconfirm suitability after substantive changes in process, formulation, equipment condition, or cleaning chemistry.

6. Pre-analytical technical gaps that can mask allergen presence

Even with the right sampling point and timing, extraction and interpretation determine whether an allergen is detectable.

6.1 Protein solubility and limits of extraction

Rapid immunoassays can only detect what they can extract and what their antibodies can recognize. In highly processed foods (roasted, baked, extruded, fermented), proteins may be denatured, become insoluble, or have altered epitopes - so a finished product can test negative even when allergen material is present.

Practical implication

For cleaning verification and cross-contact prevention, environmental verification (high-risk swab sites) and ingredient-focused controls are often more reliable than relying only on testing a highly processed finished product. Finished-product testing can add value in the broader program, but should not be used as the primary evidence for cleaning effectiveness. Validation of the detection method within these particular processed matrices enhances the reliability of the results.

Complex matrices can further reduce recovery (e.g., extreme pH, polyphenols/tannins, high fat). Where method instructions allow, validated adjustments (e.g., clarification steps) may improve recovery.

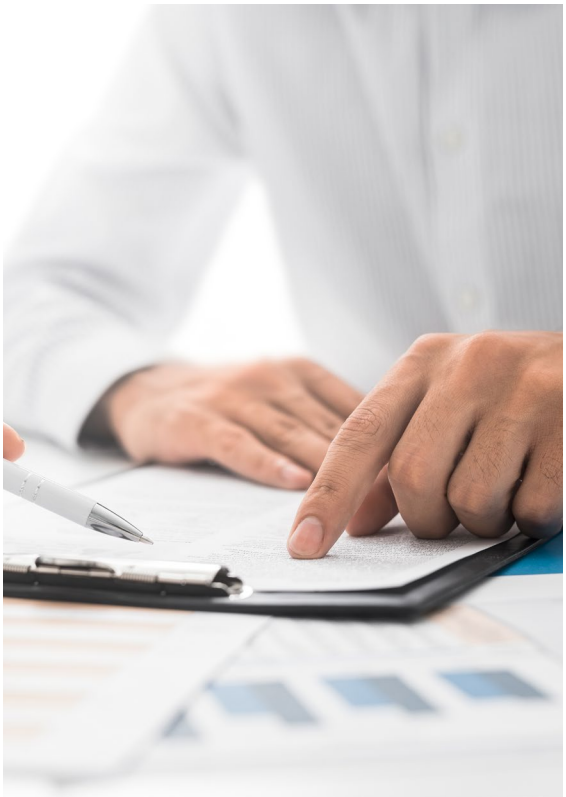
6.2 Sampling representativeness: grab vs. incremental / composite approaches

When contamination is heterogeneous, single-increment (“grab”) sampling provides limited confidence. Incremental and/or composite approaches can improve representativeness when designed and documented appropriately.

6.3 Interpretation pitfalls: cross-reactivity and non-ideal matrice

Most kits document **cross-reactivity**. Ignoring it can lead to misinterpretation - especially in multi-ingredient foods.

For rinse water and post-sanitation surfaces, **detergents** and **sanitizers** can influence assay performance. If cleaning chemistry changes, consider matrix/surface verification to confirm ongoing method suitability.



7. Common failure modes (and corrective actions)

Below are frequent ways allergen programs fail before the assay begins - and corrective actions that reduce false confidence.

Failure mode 1: Sampling convenience, not risk

Corrective action: Prioritize harborage points (seals, gaskets), transfer points, worn belts, dead legs/low-flow zones, powder dust zones. Define these as unique test sites with rationale.

Failure mode 2: Wrong timing (missing the transfer event)

Corrective action: Time verification to the decision - especially pre-op for line release. If idle-time recontamination is plausible, verify closer to start-up and control exposure (covers, restricted access, zoning).

Failure mode 3: Cross-contamination during sampling

Corrective action: Enforce disciplined technique - fresh gloves, controlled containers, single-use tools where feasible, avoid touching non-target surfaces, and define handling steps in the SOP.

Failure mode 4: Not sampling intermediates during investigation

Corrective action: Add intermediate sampling at key steps to localize sources and shorten investigations.

Failure mode 5: Over-reliance on negatives from highly processed matrices

Corrective action: For baked/extruded/fermented products tested by antibody-based methods, recognize extraction/epitope risks. Position environmental verification and ingredient controls as primary barriers where appropriate.

8. Operational realities that belong in the SOP

8.1 Swab recovery varies by surface

Stainless steel is generally cooperative. Scratched plastics, porous materials, worn seals, and rough welds are not. Proteins can lodge in micro-scratches or pores where swab recovery is reduced even though product contact still occurs.

SOP controls

- define swab area (template where feasible) and standardize pressure/pattern
- prioritize worst-case materials and worn zones, not only smooth panels
- use the validated swab/buffer system (do not improvise)

8.2 Sanitizer residues can influence results

If surfaces carry residual sanitizers (e.g., quats, peracetic acid), those chemicals may affect extraction and/or test performance. Do not assume different swab systems are equivalent.

SOP controls

- use swab systems/buffers validated for post-sanitation conditions
- specify adequate rinse/dry time where required
- document suitability and re-check if chemistry changes

8.3 Sample handling: time, temperature, containment

Between collection and extraction, sample integrity can be compromised or contamination introduced.

SOP controls

- define maximum hold time before extraction/testing
- define transport/storage temperature (as method-appropriate)
- secure containment to prevent leakage/contact contamination
- document chain-of-custody steps during investigations

8.4 Process controls for sampling integrity (high audit value)

Add simple controls that detect sampling process failures:

- Field blank swab: handled like a real swab but does not touch a surface (detects handling contamination).
- Periodic competency checks: verify that operators can execute the defined technique consistently.
- Lot-change bridging / comparability: when changing kit lots or switching kit types document suitability for your matrices/surfaces.

9. Results in context - decision rules, thresholds, and program intent

A detection result is a data point, not an automatic recall decision. But decision rules must match program intent.

9.1 Routine cleaning verification decision rule (environmental swabs)

For routine verification used for pre-op/line release, decision rules should be conservative and simple:

- apply the kit's validated interpretation rules (including controls and any matrix/surface limitations).
- For defined line-release sites, treat any confirmed detection as a failure at that site, triggering predefined corrective actions (commonly recleaning + resampling + documentation + escalation if repeated).

9.2 Product and label decision frameworks (separate from cleaning verification)

Some businesses compare analytical outcomes in products to a documented risk assessment framework (e.g., reference dose / action level concepts) to support consistent decisions around precautionary labeling and risk communication.

This is separate from cleaning verification and requires:

- method capability understood for that matrix (recovery, interferences, detection/quantitation limits),
- alignment with internal policy, customer requirements, and applicable regulations
- clear documentation of assumptions and decision thresholds.

9.3 Indicator tests (ATP / total protein): useful, but not allergen-specific

ATP and total protein can be valuable for hygiene verification and trending, but **they do not confirm allergen absence** and **should not replace allergen-specific verification** at critical sites - especially for allergen-free line release decisions.

10. A practical workflow that stands up operationally and in audits

Incoming controls

- Screen high-risk raw materials with appropriate methods where relevant
- Use supplier documentation strategically, not blindly
- Define escalation and containment actions for suspect material

Method and matrix/ surface suitability checks (before relying on results)

- Confirm the method performs as expected in your matrices and on your surfaces using appropriate controls and documented procedures
- When processes, formulations, equipment condition, or cleaning chemistries change, reassess suitability

Cleaning validation studies (periodic, intensive)

- Worst-case runs, higher sampling density, more replicates
- Multiple unique sites/materials (including disassembly points where feasible)
- Pre-defined acceptance criteria and revalidation triggers

Routine cleaning verification (everyday, targeted)

- Pre-op/line release swabs at defined unique high-risk sites (Zone 1 and key Zone 2)
- Rinse-water checks where swabbing is impractical (with chemistry/ dilution compatibility considered)
- Clear rule set for positives (corrective action + re-verification + escalation)

Finished product testing (positioned correctly)

- Can support broader program confidence and investigations
- Should not be treated as primary cleaning validation/verification evidence due to representativeness, dilution, and processing effects

The objective is not indiscriminate testing; it is risk-based testing designed so that results are actionable and defensible.

11. Checklist - design principles that prevent false confidence

- ☐ Sample where allergen residues can persist and transfer - not where sampling is easiest.
- ☐ Separate validation (can work) from routine verification (did work today).
- ☐ Define unique test sites rigorously (material, geometry, cleaning method, exposure, transfer risk).
- ☐ Use allergen zoning to justify and prioritize sites (Zones 1 - 2 first; Zone 3 for dust/traffic control where relevant).
- ☐ Time verification to the decision point - especially pre-op/line release - and control idle-time recontamination risks.
- ☐ Standardize technique (area, pressure, pattern, handling) to reduce variability.
- ☐ Prevent cross-contamination during sampling with controlled technique and handling.
- ☐ Treat sample preparation as part of the method; recovery depends on it.
- ☐ Respect matrix and chemistry effects (processing, pH, polyphenols/tannins, detergents/ sanitizers).
- ☐ Interpret results with cross-reactivity and method capability in mind.
- ☐ Use indicator tests (ATP/total protein) appropriately - but do not treat them as allergen clearance evidence.
- ☐ Use finished product testing thoughtfully - but don’t substitute it for direct evidence of cleaning effectiveness.

About Romer Labs

Romer Labs supports food producers with practical, fit-for-purpose allergen testing solutions designed to reduce the risk of unintended allergen presence across the production process. Our approach is built around helping teams generate reliable, defensible results - not only through analytical performance, but by enabling consistent sampling and routine execution within HACCP-aligned allergen management programs.

Our allergen portfolio includes qualitative and quantitative tools to match different decision points and operational realities. AgraStrip® Pro allergen lateral flow devices provide rapid, on-site screening, while AgraQuant®Pro allergen ELISAs enable quantitative assessment where measurement and documentation requirements call for it. These tests support common plant needs such as raw material and finished product checks, rinse-water testing to inform cleaning validation activities, and environmental swab testing to verify cleaning effectiveness at defined high-risk sites.

In addition to test kits, Romer Labs provides supporting resources - including reference materials and analytical services - so teams can build confidence in method suitability and maintain consistent performance over time. With deep experience in food allergen testing, Romer Labs helps customers implement allergen verification workflows that are actionable on the floor and defensible when results matter most.

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