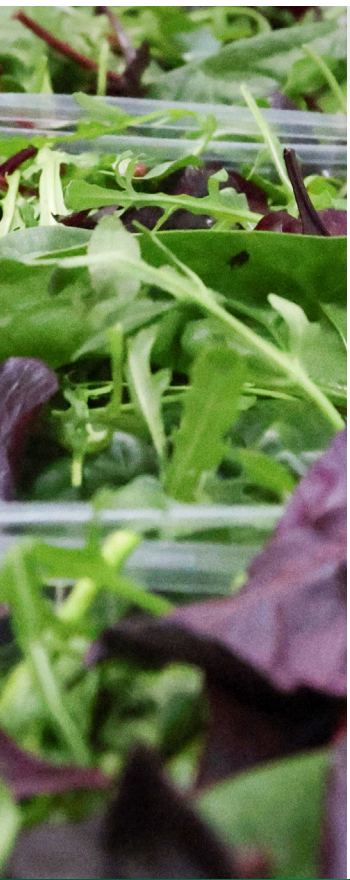
NEOGEN®

Risk-Based Food Safety Handbook

for the Food and Beverage Industries



1st Edition

This handbook provides essential information and tools to implement a **risk-based** food safety management system in your organization.

A **risk-based** food safety management system is:

1. **Holistic**, by evaluating, managing, and communicating potential food safety risks along the entire food chain.
2. **Proactive**, by anticipating potential risks and avoiding unintended consequences.
3. **Optimized**, by allocating resources to maximize food safety protection.

Risk-Based Food Safety Handbook

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Throughout this handbook you will find several activities to better understand the concepts described and how they may be used in your own facility.

This handbook is intended to be a helpful resource to assemble a risk-based food safety program and it may be helpful to go back to these activities in times of change or regular intervals for reassessment.

CHAPTER 1

A foundation for risk analysis

1.1 Unseen, uncounted: The true toll of foodborne illness

Unsafe food and water remain significant global public health concerns. Countries around the world monitor the incidence of foodborne diseases in the population by reporting annual cases and outbreaks. However, the true health impact of unsafe food is largely unknown due to significant underreporting.

The World Health Organization (WHO) has estimated that each year, **600 million people fall ill and 420,000 people die from unsafe food**, resulting in the loss of 33 million healthy life years (DALYs). Children under 5 years of age are at particularly high risk, with 125,000 children dying from foodborne diseases every year.

These estimates, along with the scientific publications, are updated every 10 years and can be accessed here:

https://www.who.int/activities/estimating-the-burden-of-foodborne-diseases

https://collections.plos.org/collection/ferg2015/

The U.S. Centers for Disease Control and Prevention (CDC) has an interactive tool with the incidence of foodborne outbreaks since 1998 (BEAM dashboard) that can be accessed here:

https://www.cdc.gov/ncezid/dfwed/BEAM-dashboard.html

The European Centre for Disease Prevention and Control (ECDC) has an interactive tool with the incidence of infectious diseases (Atlas) that can be accessed here:

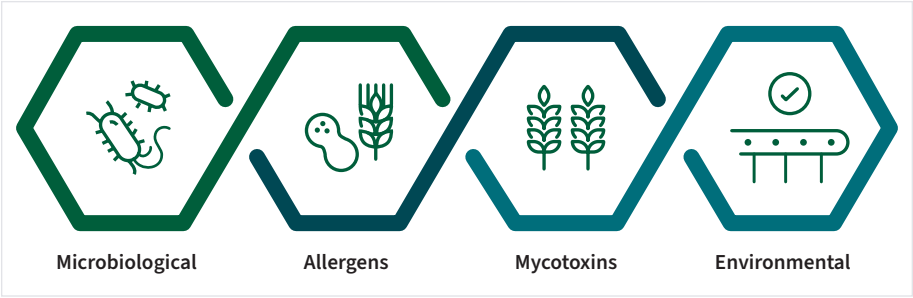
https://atlas.ecdc.europa.eu/public/index.aspx

1.2 Risk-analysis principles

1.2.1 Risk-based food safety program

A comprehensive, risk-based food safety program must incorporate tools and procedures to identify, assess, and control all potential food safety hazards tailored to your products and facility. It should also address food quality risks that may impact business profitability and brand credibility.

Figure 1. Risk-based food safety program



By undergoing a full risk-analysis process, a food safety and quality program transforms. **This fundamental shift from a reactive to a preventive system is crucial — transitioning the industry from relying on end-product testing to focusing on preventing contamination at its source**.

It is important that food companies conduct a proper risk assessment tailored to their food products and facilities, *not approach it as a generic* "copy and paste" exercise. It requires:

- Deeply understanding the hazards and factors that contribute to the risk.
- Allocating resources appropriately to prevent the risks and their root causes.
- Truly evaluating the effects of risk management efforts.

1.2.2 Hazard vs. Risk

Hazard and risk are not interchangeable terms; they mean different things.

Hazards are agents that cause negative health effects (short and long-term). For example, microbial hazards are short-term (hours or days), and chemical hazards are long-term (years). Microbial indicators are not hazards, but they can indicate the presence of a pathogen or affect food quality. *For more information on microbial indicators, see Section 3.1.1.*

Risk is the consequence of exposure to a hazard. For example, allergens have threshold doses that trigger an immune reaction; the sole presence doesn't equal risk.

Food safety risk is expressed as a combination of:

RISK = EXPOSURE x SEVERITY

Exposure

Likelihood of a hazard to be present in a raw material or food product. It is usually quantified by the contamination rate or prevalence (%). *See Section 4.1.2 on how to estimate the contamination rate.*

Sources of information:

- Industry-owned data
- National recalls — e.g., FDA and USA recall database
- International alerts — e.g., FDA and RASFF EU import rejects
- National sampling plans
- International guidelines
- Scientific published studies

See Section 3.2 for sources of information for exposure.

Severity

Magnitude of the adverse health effect that can result from exposure to a food safety hazard.

Sources of information:

- Incidence of foodborne diseases — e.g., hospitalization and mortality rates
- Toxicological substance profile — e.g., potential carcinogenic by International Agency for Research on Cancer (IARC)
- Health effects of a chemical substance — e.g., Agency for Toxic Substances and Disease Registry (ATSDR)
- Allowable Daily Intake (ADI) reference values — e.g., Codex reference values for pesticides and veterinary drug residues

See Section 3.2 for sources of information for severity.

If you are interested in learning more on how to conduct a risk assessment, please see Chapter 3.

CHAPTER 2

Basic principles of food sampling

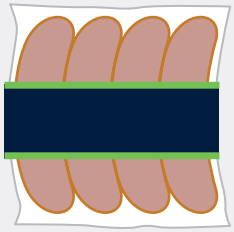
2.1 How reliable is finished product testing?

Food safety management systems have traditionally relied on the testing of the finished product as a verification activity to ensure that a lot or batch is free of microbial and chemical contamination and thus is safe for consumption.

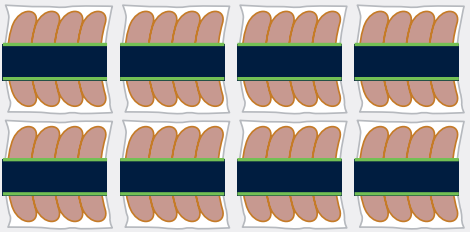
2.1.1 Basic principles of sampling

1	One can only be 100% sure that the lot is free of contamination if all the units in that lot are analyzed.
2	Microorganisms are often heterogeneously distributed in a lot due to several reasons like heterogeneity of the food matrix and incomplete mixing.
3	The higher the number of samples taken from a lot, the higher the probability of detecting a contaminated lot.
4	The higher the number of contaminated units in a lot, the higher the probability of detecting a contaminated lot.
5	Sampling represents a very small fraction of the lot. If the sampling result is positive, this means that the lot is contaminated. If negative, it does **not** guarantee that the lot is free of microbial contamination.

Figure 1. Definitions of unit and batch



Unit: Individual package that contains a portion size.

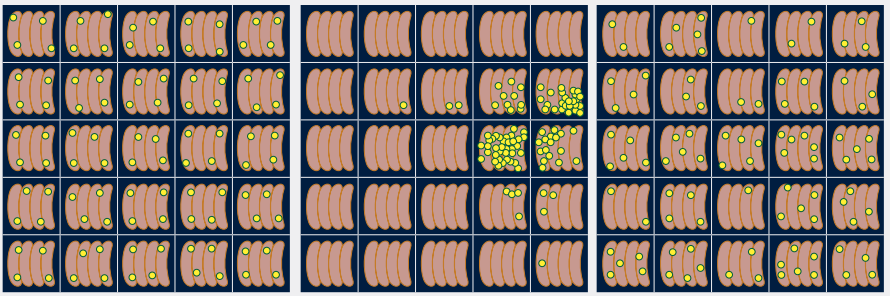


Lot or batch: Amount of product manufactured under the same conditions.

Figure 2. Heterogeneity of microorganisms distributed in a food matrix

In grid, each square is one unit in the lot:

= microorganisms



Homogeneous — not realistic

Heterogeneous

Heterogeneous

Source: ILSI Europe. Impact of Microbial Distributions on Food Safety.

2.1.2 Probability of detecting a contaminated lot

One can ask the following question: How reliable is the testing scheme to be able to detect a contaminated lot in my company? There is a tool to answer that question. **The gold standard of any sampling plan is reaching 95% level of confidence of detecting a contaminated lot.**

Table 1. Example of probability of detecting a contaminated lot

Units in a lot	Contamination rate	Units contaminated in a lot	Samples analyzed	Probability of detecting
1,000	1%	50	1	**1%**

Source: Zwietering et al., 2015.

Extremely low (1%)!

Units in a lot is the number of packages or cans in your actual lot.

Contamination rate is the percentage of units contaminated in the lot, an estimation based on historical records or published information.

Samples analyzed is the current final product testing plan of your product (e.g., one sample per lot).

As shown in the example, when the contamination rate is low (e.g., 1%) and one sample is analyzed per lot, the probability of detecting a contaminated lot is **extremely low (1%)**! If only one sample is sampled from a lot, almost all the lot needs to be contaminated (95% contamination rate) to detect a contaminated lot with a 95% level of confidence.

Activity 1: Calculate the probability of detecting a contaminated lot

Use the table below to calculate the probability of detecting a contaminated lot based on your own food characteristics and testing scheme.

1 Units in a lot	2 Contamination rate	Units contaminated in a lot	3 Samples analyzed	Probability of detecting
	%			%

1. Enter the number of packages or cans in your actual lot.
2. Enter the percentage of units (whole number) contaminated in the lot, an estimation based on historical records or published information.
3. Enter the number of samples tested.

Answer these two questions:

Based on your current testing, what is the probability of detecting a contaminated lot?

How many samples should you need to test to achieve a 95% probability of detecting?

Reset Form

CASE STUDY 1

Effectiveness of finished-product sampling to detect *Listeria monocytogenes* in hot dog lots

A ready-to-eat hot dog plant produces large lots (tens of thousands to millions of units). *Listeria monocytogenes* contamination, when it occurs, typically affects only a fraction of finished units within a lot (e.g., post-lethality contamination of some packages). This case study illustrates the probability of detecting a contaminated lot using finished-product sampling under different sampling designs.

ASSUMPTION: Each sampled unit is effectively a random draw from the lot, and the chance of detection depends on the contamination prevalence in the lot and how many units you test.

Scenario 1

50,000 units per lot
Contamination from 0.1% to 26%
10 samples per lot

Key point: with only **10 samples**, you need roughly **~26%** of the lot contaminated to reach **~95%** confidence of detection.

Interpretation: 10 samples per lot is unrealistic and very costly, and it will only detect a contaminated lot under a very dramatic contamination event.

Scenario 2

50,000 units per lot
Contamination from 0.1% to 10%
Sample size increased (~28 up to ~2,900+)

Key point: Even at **10% contamination** (5,000 contaminated units-catastrophic), you need **~29 samples** per lot to get to ~95% confidence.

Interpretation: Even at 10% of contamination rate, the number of samples to be taken from a lot are totally unrealistic; at lower prevalence, the sample counts explode into the hundreds or thousands.

Scenario 3

10,000 to 1,000,000 units per lot
Contamination = 0.01%
One sample per lot (routine daily testing)

Key point: At **0.01% prevalence**, testing **one unit** gives a detection probability of **0%**!

Interpretation: Routine “one sample a day” pathogen testing will miss almost all contamination lots *unless a catastrophe occurs*.

CASE STUDY 1 continued

Figure 3. Hot dog lot sampling scenarios

Scenario 1

As the number of samples contaminated in a lot increases, the probability of detection increases as well.

We need to have at least 26% contaminated units in a lot to be able to detect that lot with 95% confidence by only taking 10 samples.

Units in a lot	Pathogen contamination	Contaminated units	Samples analyzed	Probability of detecting
50,000	0.001 (0.1%)	50	x 10	1%
	0.01 (1%)	500		10%
	0.05 (5%)	2500		40%
	0.1 (10%)	5000		65%
	0.26 (26%)	13000		95%

Scenario 2

As the percentage of pathogen contamination increases, the number of samples needed to be analyzed in a lot to be 95% confident is reduced.

Even with 10% contamination level (5,000 units contaminated, a total disaster!), we need to take 28 samples in each lot to be 95% confident that we can detect that lot.

Units in a lot	Pathogen contamination	Contaminated units	Samples analyzed	Probability of detecting
50,000	0.001 (0.1%)	50	x 2,900	95%
	0.01 (1%)	500	x 300	
	0.05 (5%)	2500	x 60	
	0.1 (10%)	5000	x 28	

Scenario 3

With a realistic very low contamination level in a lot (0.01%) from a processed food product and the routine testing of one sample daily for a pathogen, the chance of detecting a lot is shockingly 0%!

Units in a lot	Pathogen contamination	Contaminated units	Samples analyzed	Probability of detecting
10,000	0.0001 (0.01%)	1	x 1	0%
50,000	0.0001 (0.01%)	5		
100,000	0.0001 (0.01%)	10		
1,000,000	0.0001 (0.01%)	100		

CASE STUDY 2

Effectiveness of finished-product sampling for detection of undeclared milk in a dairy-free bakery

A bakery produces cookies labeled as “dairy free.” The production line also handles milk-containing products on other days. Despite sanitation procedures, there is a credible risk of unintended milk cross-contact, resulting in some finished units within a lot containing milk protein. The purpose of this case study is to illustrate the probability of detecting undeclared milk at the lot level using finished-product sampling under different sampling designs. The lot contains a very large number of cookies. Milk contamination, when present, affects only a fraction of cookies within the lot.

Table 2. Cookies lot sampling scenarios

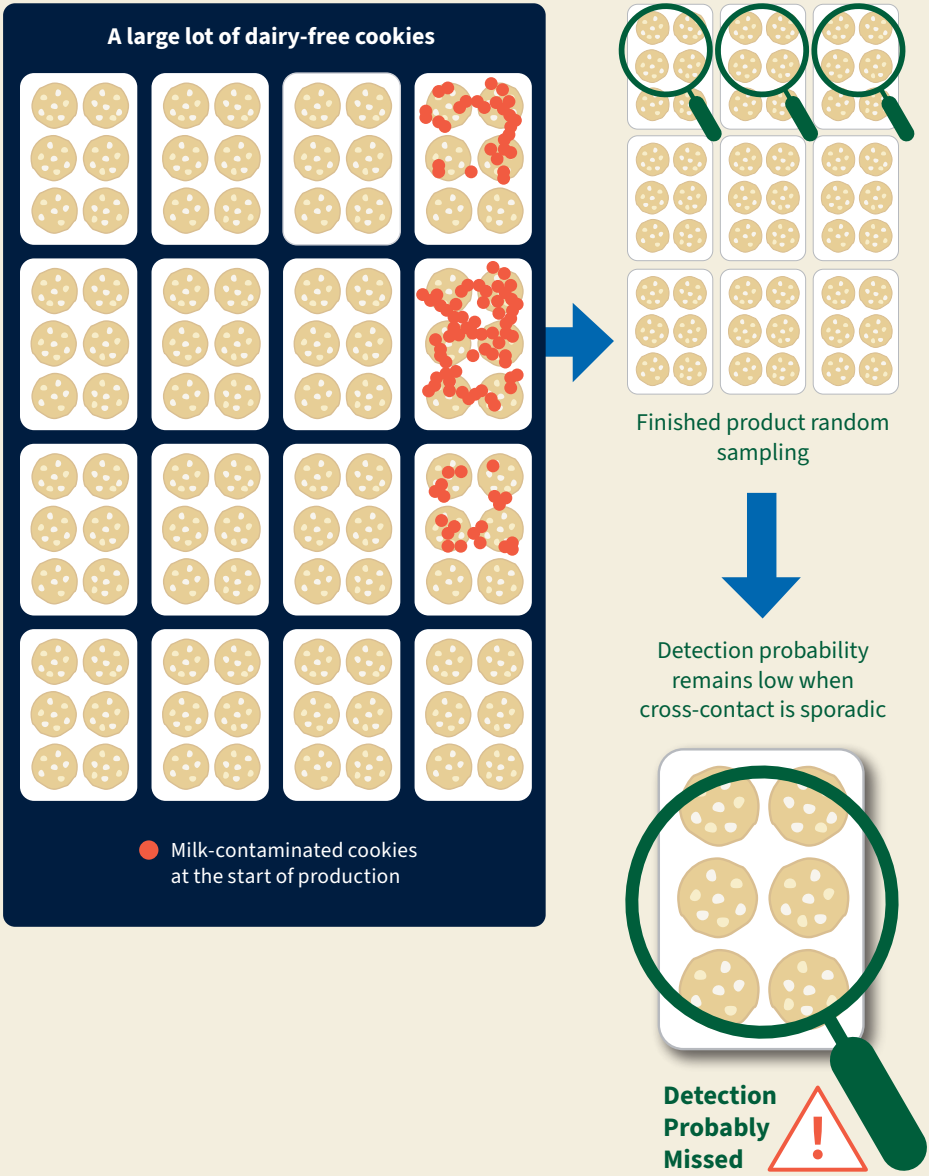
Units in a lot	Proportion of cookies containing milk	Milk-containing units	Samples analyzed	Probability of detecting a lot containing milk
10,000	0.1%	10	1	**0.1%**
	0.1%	10	5	**0.5%**
	1%	100	1	**1.0%**
	1%	100	5	**5.0%**
	5%	500	1	**5.0%**
	5%	500	5	**22.6%**

Even at a contamination rate of 1%, the likelihood of detecting undeclared milk remains extremely low. Increasing sampling from one to five units per lot improves detection probability, but the improvement is modest. When contamination prevalence is low, as is typical for allergen cross-contact events, the probability of detection remains well below levels commonly interpreted as providing confidence.

Overall, the case study demonstrates that **finished-product testing alone is not an effective control for undeclared allergens** and primarily detects only severe or catastrophic contamination events. Preventive controls and validated sanitation remain the primary risk-management tools.

CASE STUDY 2 continued

Figure 4. Cookies lot sampling scenarios



2.1.3 Role of composite sampling

Microbiological criteria regulations (*see Section 3.1.5*) require testing multiple food samples for certain pathogens like *Salmonella* spp. or STEC. To save resources, many labs and companies use compositing and/or pooling replicate samples. Though cost-effective and efficient, compositing may impact detection sensitivity and accuracy, so careful evaluation is necessary to maintain food safety.

Understanding these terms is essential to ensure consistent application and interpretation of compositing practices:

Composite sample — mixed sample of a number of the same items of food, animal feed, animals, or environment, from which **a test portion is taken** for examination in the laboratory.

Pooled sample — mixed sample of a number of the same items of food, animal feed, animals, or environment, where the **complete mixture** is the test portion and is taken as a whole for examination in the laboratory.

Key aspects of compositing/pooling:

- **Statistical validity:** If a method meets required accuracy, precision, and reliability standards, the composite approach should be statistically consistent with the individual testing method. For instance, testing 30 x 25 g units is as valid as testing 3 x 250 g units, since both yield equal sample mass and capture population variability.
- **Sensitivity requirements:** Only use compositing if the method can detect low levels of target organisms in the pooled volume. For instance, if 1 CFU in 25 g is required and you combine ten 25 g samples (250 g total), the method must reliably find 1 CFU in the whole 250 g. If sensitivity decreases with larger samples, low-level contamination may be missed, causing false negatives. Validate that detection capability remains at higher volumes before compositing.
- **Quantitative vs. qualitative:** Compositing is *only* appropriate for qualitative (presence/absence) analysis. It should not be used for quantitative (count-based) tests. Pooling dilutes bacteria, potentially causing false negatives or nonconforming results and making it harder to identify the contamination source in production.
- **Recall/outbreak:** In the event of a recall or outbreak, using pooling can make it much harder to pinpoint the specific contaminated lot that needs to be recalled. This difficulty can lead to broader recalls, increased costs, and greater disruption for suppliers and consumers.

The International Commission on Microbiological Specifications for Foods (ICMSF) (2002) provides guidelines on using sampling inspection plans for food quality/safety assurance.

Example of ICMSF sampling plan and economic impact

Table 1. Numbers of samples (of specified size) that must be tested from a lot (with homogeneous contamination) *and shown not to be contaminated* to ensure that the average contamination with *C. botulinum* in the lot is less than 1 CFU per 100 g. The table shows the effect of sample size and rigor of testing (expressed as confidence that the result is accurate).

Sample size	Confidence in result of testing	Number of samples required*	Total cost @ $150/sample	8 sampling time points**
1 gram	95%	299	$44,850	$358,800
10 gram	95%	29	$4,350	$34,800
25 gram	95%	11	$1,650	$13,200
	99%	17	$2,550	$20,400
	90%	9	$1,350	$10,800

*Samples are typically pulled and analyzed at multiple time points.
**Cost is for illustrative purposes only.

To demonstrate (with 95% confidence) that the batch satisfies the criterion requires > 180 samples of 1 g to be tested, and none to be found positive. This is a significant number of samples. The table above outlines the economic impact.

The ISO 6887-1 standard outlines four types of situations where compositing or pooling may be valid. All these procedures must be verified before use to show that the risk of false-negative results has not been increased.

Figure 5: Composite
Items are composited into one sample and mixed before the test portion is taken.

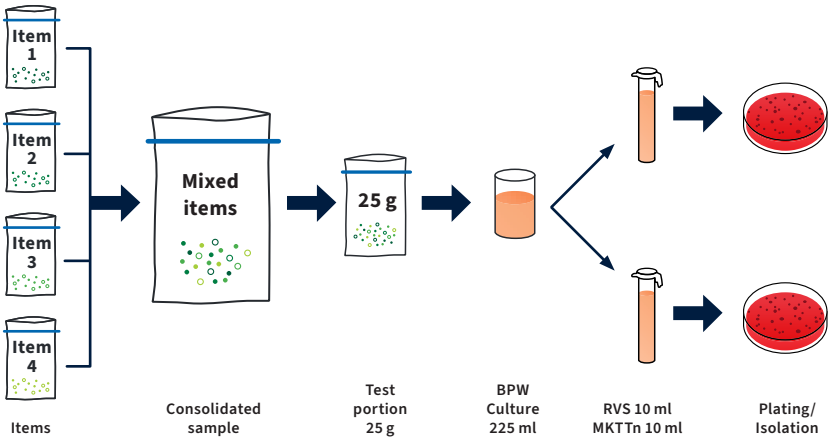


Figure 6: Pooled (dry pooling)
Items of the same type are pooled into one sample and the whole mixture is used as the test portion.

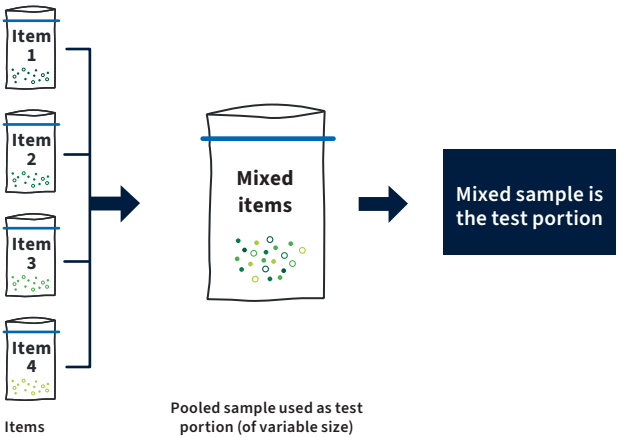


Figure 7: Pooled (wet pooling)
The test portions from a number of items of the same type are mixed and the whole mixture is used as the test portion.

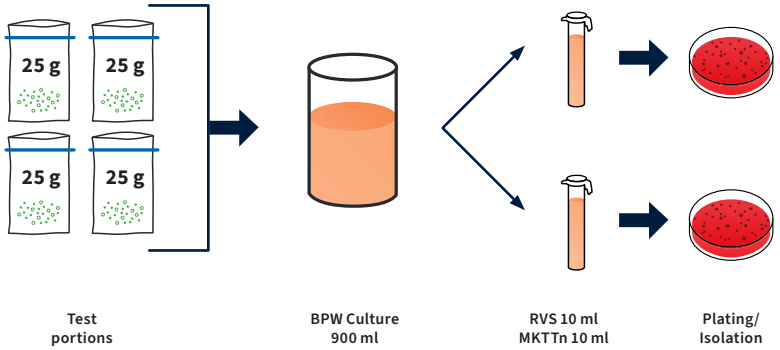
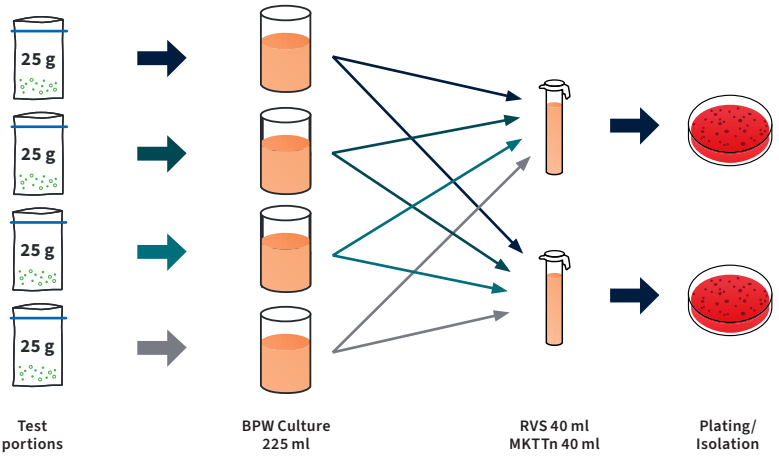


Figure 8: Pre-enriched pooled
The test portions of several items of the same type are pre-enriched, and then a specified volume from each culture is combined.



Learn more about sample collection solutions

Connect with a Food Safety expert

CHAPTER 3

Risk assessment

3.1 Hazard identification

Hazard identification is the first step in a food safety risk assessment process. To conduct this, look for sources of information to identify potential food safety hazards (e.g., pathogens, allergens, mycotoxins).

3.1.1 Microbial indicators

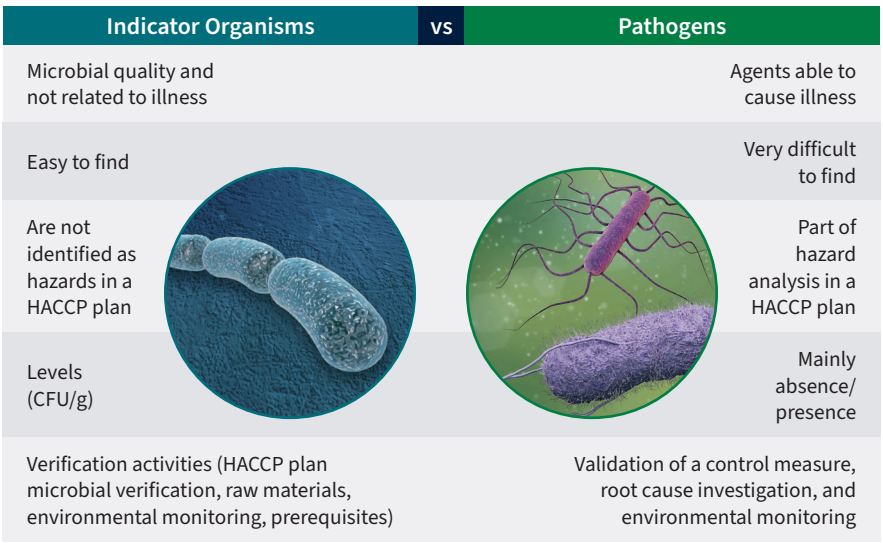
Microbial indicators are used as a verification means of the quality, hygiene, or environmental microbial niches of raw materials, surfaces, utensils, hands, and final product.

Microbial indicators *are not hazards*; they don't produce illness and thus are not part of the hazard identification or critical control point (CCP) validation studies in the HACCP plan.

They are very useful in the verification activities of your food safety plan. They need to be *quantified*.

CAVEAT: If you never see quantifiable levels in an ingredient, product, or surface (*always absence* for let's say several years), it is a sign that is not a suitable indicator. So, you need to look for another indicator — or the ingredient is not prone to microbial contamination.

Figure 1. Main differences between pathogens and indicators



A useful way to identify suitable microbial indicators is to think about what the purpose of testing is — i.e., why am I testing for this indicator?

Figure 2. Type of Indicators

Quality	Hygiene	Environmental
Used to evaluate the potential damage of the quality of your product (change of color, aspect, flavor, etc.).	Used to evaluate the potential for fecal matter contamination, lack of hand washing, or excessive manipulation.	Used to check the cleanliness of a surface or equipment or to avoid recontamination of the product.
Example: LAB, YM, AC	**Example:** Generic *E. coli*, *S. aureus*, EB	**Example:** AC, *Listeria* spp., YM, EB

CAVEAT: If the answer is either "because my customer requires it" or "we always have tested for it," you should carefully review your sampling plan and have a technical reason for your decisions.

For example, generic *E. coli* has been recommended as the most suitable indicator for the presence of fecal contamination in irrigation and water used in fresh produce packing plants as an indicator for the possible presence of pathogenic *E. coli*.

Figure 3. Main hygiene indicators

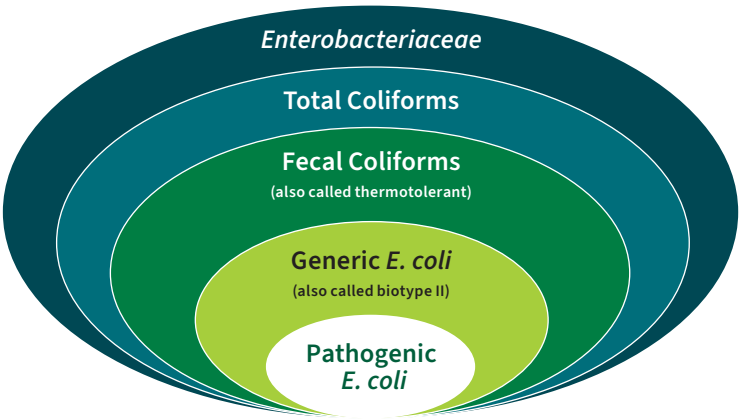


Table 1. Main microbial indicators and their intended use

Indicator organism	Primary purpose	Typical applications	Technical justification
Aerobic Plate Count (APC)/Total Viable Count (TVC)	Overall process hygiene and trend monitoring	Raw materials, in-process control, finished products, shelf-life studies	Non-specific indicator of microbial load used to detect loss of process control and monitor trends over time; not a direct food safety indicator
Enterobacteriaceae	Hygiene and process control indicator	Raw materials, RTE foods, environmental monitoring	Broader and more sensitive hygiene indicator; correlates better with control of enteric pathogens
Generic *Escherichia coli*	Fecal contamination indicator	Raw foods, water, fresh produce, meat and poultry	Strong indicator of fecal contamination; more predictive of *Salmonella* or STEC risk than total coliforms
Psychrotrophic plate count	Cold-chain and refrigeration control	Refrigerated and extended shelf-life foods	Detects microorganisms capable of growth at refrigeration temperatures; useful for spoilage and *Listeria* risk trending
***Listeria* spp. (non-monocytogenes)**	Environmental hygiene indicator	RTE facilities, environmental monitoring (Zones 2–4)	Indicator of conditions that could support *Listeria monocytogenes* presence; widely used in EMPs
Yeasts and molds	Spoilage and sanitation effectiveness	Acidified foods, bakery, dairy, beverages	Indicates environmental hygiene and shelf-life stability
Sulphite-reducing clostridia/*Clostridium* spp. (indicators)	Anaerobic hygiene and spore control	Canned foods, vacuum-packed or reduced-oxygen products	Conservative indicators of inadequate thermal processing or anaerobic control
Process-specific surrogate organisms (e.g., *Listeria innocua*, *Enterococcus faecium*, *Bacillus* spores)	Validation and verification of lethality steps	Thermal, non-thermal, or antimicrobial interventions	Surrogates allow safe, practical validation of kill steps where pathogen testing is impractical or unsafe

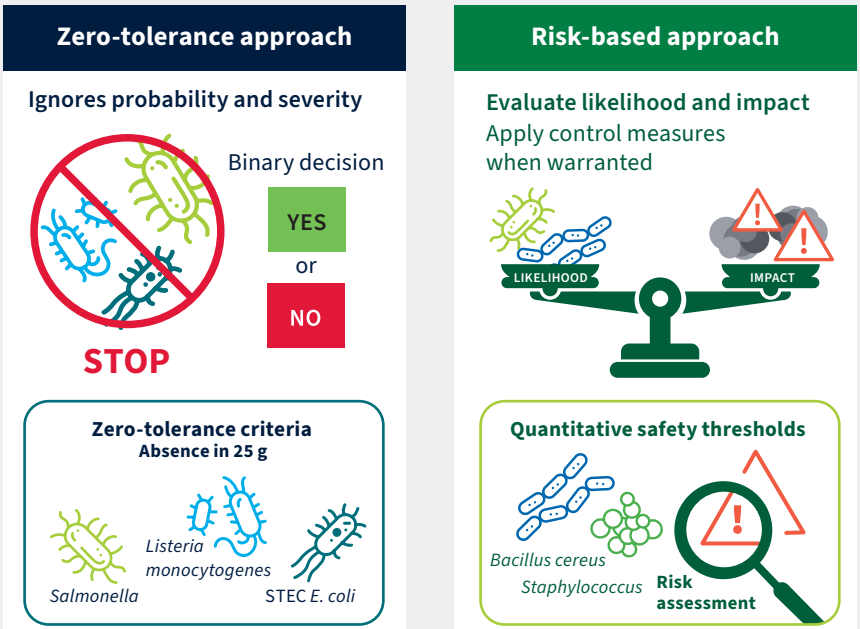
3.1.2 Pathogens

Assuming "zero tolerance" often reduces hazard identification to a binary yes/no decision, where any risk is considered unacceptable and probability and severity are effectively ignored. A true risk-based approach instead recognizes that both likelihood and impact matter, requiring food safety professionals to weigh these factors before deciding whether preventive controls are warranted.

Many severe microbial hazards (e.g., *Salmonella* spp., *Listeria monocytogenes*, STEC *E. coli*) are regulated under zero-tolerance criteria (absence in 25 g), while others (e.g., *Staphylococcus aureus*, *Bacillus cereus*) have defined quantitative safety thresholds. Recent regulatory and scientific trends increasingly favor risk-based control strategies like the use of quantitative safety thresholds for *Listeria monocytogenes* in low-risk products that do not support growth, and the use of quantitative criteria and the distinction of public-health serotypes from those of animal-health relevance for *Salmonella* in poultry.

Figure 4. Addressing pathogens in food safety

Zero-tolerance reduces hazard identification to binary decisions, ignoring probability and severity. A risk-based approach evaluates likelihood and impact to tailor preventative controls, often using quantitative safety thresholds.



3.1.3 Mycotoxins

Mycotoxins are chemical substances produced by fungi, including molds, yeasts, and mushrooms when conditions such as temperature, humidity, and nutrients are favorable. These toxins can develop before harvest or during storage, particularly if grains are mishandled. Mycotoxins themselves cannot be destroyed by heat or freezing, and there are no reliable methods for neutralizing them without damaging the grain (e.g., ammonization is effective for aflatoxins but affects kernel quality). Some mycotoxins like aflatoxins are highly toxic to humans and animals. Additionally, fungal contamination can reduce grain quality and nutritional value. *Annex 1 shows the maximum levels for mycotoxins in food*.

Table 1. Main types of mycotoxins and food sources

Mycotoxin	Main types	Producing fungi	Main food sources
Aflatoxins	Aflatoxin B1 (most toxic), B2, G1, G2, M1 (in milk)	*Aspergillus flavus, Aspergillus parasiticus*	Corn and corn products; peanuts and other nuts (pistachios, almonds); cottonseed; spices (e.g., chili, turmeric); milk and dairy products (aflatoxin M1 after carry-over in cows)
Ochratoxin A (OTA)	Ochratoxin A	*Aspergillus ochraceus, Penicillium verrucosum*	Cereals (wheat, barley); coffee beans; dried fruits; wine and grape juice; spices
Fumonisins	Fumonisin B1 (most prevalent), B2, B3	*Fusarium verticillioides, Fusarium proliferatum*	Maize (corn) and corn-based products
Deoxynivalenol (DON, vomitoxin)	Deoxynivalenol	*Fusarium graminearum, Fusarium culmorum*	Wheat; barley; oats; corn
Zearalenone (ZEA)	Zearalenone	*Fusarium graminearum, Fusarium culmorum*	Maize; wheat; barley; sorghum
T-2 and HT-2 toxins	T-2 toxin, HT-2 toxin	*Fusarium sporotrichioides*	Oats; barley; wheat; corn
Patulin	Patulin	*Penicillium expansum*	Apples and apple products (juice, purée); pears and stone fruits; rotten or bruised fruit

3.1.4 Allergens

Allergens are chemical hazards that can cause rapid, severe reactions such as IgE-mediated anaphylaxis, which is why they are regulated. Chronic responses like celiac disease and lactose intolerance are relevant but typically do not require mandatory labeling. For reference, products must have less than 20 ppm gluten to be labeled "gluten free," under 100 ppm lactose for "lactose free," and any food containing 10 ppm or more sulphites* must declare it on the label.

Table 2. Allergens that need food safety controls and mandatory labeling
Source: FAARP Nebraska

CODEX	EU	USA	Australia/ New Zealand	Japan	Canada
Milk	Milk	Milk	Milk	Milk	Milk
Eggs	Eggs	Eggs	Eggs	Eggs	Eggs
Peanuts	Peanuts	Peanuts	Peanuts	Peanuts	Peanuts
Soybeans	Soybeans	Soybeans	Soybeans	Walnuts	Soybeans
Cereals containing gluten (wheat, barley, rye, oats, spelt, etc.)	Cereals containing gluten (Including wheat, barley, rye, etc.)	Wheat	Cereals containing gluten (Including wheat, barley, rye, etc.)	Wheat	Wheat or triticale
Tree nuts	Tree nuts (almonds, Brazil nuts, cashews, hazelnuts, macadamia nuts or Queensland nuts, pecan nuts, pistachio nuts, walnuts)	Tree nuts (almonds, Brazil nuts, cashews, filbert/ hazelnuts, macadamia nuts, pecans, pine nuts, pistachios, walnuts [black, California, heartnut, Japanese, English, Persian])	Tree nuts (almonds, Brazil nuts, cashews, hazelnuts, macadamia nuts, pecans, pistachios, pine nuts, walnuts)	Buckwheat	Cereals containing gluten (Including wheat, barley, rye, etc.)
Fish	Fish	Fish	Fish	Crab, shrimp	Tree nuts (almonds, Brazil nuts, cashews, hazelnuts, macadamia nuts, pecans, pine nuts, pistachios, walnuts)
Crustaceans/ Molluscs	Crustaceans	Crustacean shellfish	Crustaceans		Fish
Sesame	Sesame	Sesame	Sesame		Crustaceans/ molluscs
Lupin	Lupin		Lupin		Sesame
					Soybeans
					Mustard

*Sulphites are not classified as food allergens but are regulated in a similar way as adverse reactions can occur in some individuals. Therefore, they may be listed with true food allergens in specific regulatory documents.

Table 3. Proposed Food and Agriculture Organization (FAO)/World Health Organization (WHO) allergen thresholds (ppm)

 Mustard, celery, tree nuts	 Milk, egg, peanut, sesame	 Hazelnut	 Fish, Wheat	 Soy, lupin, buckwheat	 Crustaceans
1.0	2.0	3.0	5.0	10.0	200

Undeclared allergens are consistently one of the top (~35–50%, depending on year) causes of U.S. food recalls, and milk is the most common cause of recalls due to undeclared allergens.

The main reasons for allergen recalls are:

- Incorrect label or packaging applied to the product.
- Label not updated after formulation or ingredient changes.
- Allergen cross-contact during manufacturing.
- Rework or ingredient misuse.
- Labeling process control failures.
- Training and knowledge gaps.
- Specification and data management failures.

3.1.5 Food safety hazards identification

For all food safety hazards, the U.S. Food and Drug Administration (FDA) published a list of potential hazards per product category. *See Hazard Analysis and Risk-Based Preventive Controls for Human Food: Draft Guidance for Industry published: Appendix 1: Potential Hazards for Foods and Processes*.

The EU also published microbiological criteria in foods where it regulates the pathogens to control in different food categories. *See Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs.*

CASE STUDY 3

Hazard identification in ice cream with chocolate chips and almonds

Biological hazards			
Food product ingredients	***Salmonella* spp.**	***Listeria monocytogenes***	**Pathogenic *E. coli***
Ice cream	✓	✓	✓
Chocolate chips	✓	✓	✓
Almonds (nuts)	✓	✓	✓

Chemical hazards			
Food product ingredients	**Drug residues**	**Mycotoxins**	**Heavy Metals**
Ice cream	✓		
Chocolate chips		Ochratoxin	Cadmium lead
Almonds (nuts)		Aflatoxin	

Allergens		
Allergen	**Potential presence**	**Technical reason**
Milk	✓	Already included as an ingredient
Egg		
Peanut	✓	Potential cross-contact with other formulations
Tree nuts	✓	Already included as an ingredient (almonds) Potential cross-contact with other tree nuts from other formulations
Wheat	✓	Cross-contact with other formulations that use cones
Soy		
Fish/crustaceans		
Sesame		

Activity 2: Identify potential food safety hazards (biological, chemical, and allergens)

List all the potential food safety hazards in your food product according to **FSMA Appendix 1** and **EU regulation**.

Food product ingredients	Biological hazards	Chemical hazards	Allergens	Technical reasons

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3.2 Likelihood and severity

Once all potential hazards have been identified, the next step in the risk assessment is to evaluate the likelihood and severity of each hazard.

Table 4. Sources of information for likelihood and severity

Food safety hazard	Variable	Information sources
 Pathogens	Prevalence of positive samples	Industry-owned data (last 5 years)
	Number of U.S. pathogen recalls	FDA recall database **https://datadashboard.fda.gov/oii/index.htm**
	Number of E.U. international alerts	RASFF database **https://webgate.ec.europa.eu/rasff-window**
	Incidence of foodborne disease in the U.S.	Foodborne Illness Acquired in the United States — Major Pathogens, 2019 **https://wwwnc.cdc.gov/eid/article/31/4/24-0913_article**
 Chemical hazards	Toxicological profiles (ATSDR)	Agency for Toxic Substances and Disease Registry (ATSDR **https://www.atsdr.cdc.gov/toxicological-profiles/glossary/index.html**
	Carcinogenic classification (IARC)	International Agency for Cancer Research (IARC) **https://monographs.iarc.who.int/agents-classified-by-the-iarc/**

Food safety hazard	Variable	Information sources
 Pesticides and veterinary drugs	CODEX	Maximum Residue Limit (MRL) and Acceptable Daily Intake (ADI) values for pesticides **https://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/pesticides/en/** MRL and ADI values for drug residues **https://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/vetdrugs/veterinary-drugs/en/**
 Allergens	Number of undeclared allergens events	Industry-owned data
	Number of U.S. allergen recalls	FDA recall database **https://datadashboard.fda.gov/oii/index.htm**
	FARRP	**https://farrp.unl.edu/**
	FALCPA	**https://www.fda.gov/food/food-allergensgluten-free-guidance-documents-regulatory-information/food-allergen-labeling-and-consumer-protection-act-2004-falcpa**
	Codex Alimentarius Guidelines	Codex General Principles of Food Hygiene **https://www.fao.org/fao-who-codexalimentarius** **https://www.fao.org/food-safety/scientific-advice/food-allergens/en**
	E.U. allergens	European Surveillance System on Contact Allergies (ESSCA) database **https://www.essca-dc.org/**
	Health Canada	**https://hc-sc.gc.ca**
	VITAL Program-Allergen Bureau	Standardized allergen risk assessment process for food industry. **https://vital.allergenbureau.net/**

Food safety hazard	Variable	Information sources
 Mycotoxins	Prevalence of non-compliant samples	Industry-owned data
	CODEX	General standard for contaminants and toxins in food and feed CXS 193-1995 **https://www.fao.org/fao-who-codexalimentarius**
	European Food Safety Authority-Mycotoxins	EFSA mycotoxins **https://www.efsa.europa.eu/en/topics/topic/mycotoxins**
	European Commission	Legislation on mycotoxins **https://ec.europa.eu**
	USDA Grain Inspection, Packers and Stockyards Administration (GIPSA)	USDA GIPSA **https://www.ams.usda.gov/**

Although this guide primarily focuses on biological and chemical hazards, a comprehensive risk-based food safety program must also address physical hazards. Each facility should conduct a hazard analysis to identify and evaluate any known or reasonably foreseeable physical hazards, such as metal fragments, glass, stones, plastic, or other foreign materials.

The significance of a physical hazard can vary between facilities, even when producing the same or similar products. Differences in raw materials, equipment design, processing operations, preventive controls, and maintenance programs can influence both the likelihood and severity of a hazard.

CHAPTER 4

Microbial risk assessment

4.1 Microbial risk assessment of raw materials

The first risk area in the food chain is microbial and chemical contamination of raw materials and ingredients. These can be contaminated at the point of origin from sources such as the irrigation water, soil, presence of domestic livestock and wildlife animals, and means of transport used to move the raw materials (e.g., bins, containers, trucks).

It is important to characterize the risk that raw materials can introduce into the processing by conducting a risk assessment and designing a risk-based sampling plan following these steps:

1	**Identify microbial and chemical contaminant targets**
2	**Characterize the contamination**
3	**Estimate the annual number of samples**
4	**Revise and redesign the sampling plan accordingly**
5	**Mitigation strategies**

4.1.1 Identify microbial and chemical contaminant targets

Use the hazard identification section to identify the indicator organisms, pathogens, mycotoxins, and allergens that could be tested for the microbiological verification of raw materials. Only raw materials known to pose a risk of contamination should be tested.

KEY

AC = Aerobic Count
CC = Coliform Count
EB = *Enterobacteriaceae*
EC = *E. coli* Count
EL = Environmental *Listeria*
LAB = Lactic Aid Bacteria
STX = *Staphylococcus aureus*
YM = Yeast and Mold

Food Matrix	Meat	Poultry	Milk Products	Milk Powders	Egg Products	Fish, Crustaceans	Molluscan, Shellfish	Fruit, Vegetables	Gums, Spices	Sweeteners	Grains, Cereals	Nuts
Hygiene	EC* EB* AC STX	AC EC STX	EC* EB* STX* AC** CC**	EB* STX* AC	EB* AC	EC* STX*	EC* STX*	EC* AC EB	EB		EB STX	
Quality	AC LAB YM	LAB YM	AC LAB YM	YM	AC YM CC EC	YM LAB AC CC	AC	AC LAB YM	YM AC EC CC	YM	YM LAB	YM AC CC EC EB
Environmental	AC CC EB STX	AC CC EB EL	EL	EB	EL AC	EL AC	EL	EL AC EC EB			EB YM	EB EC

Source: Compendium of Methods for the Microbiological Examination of Foods, 5th Edition, APHA and International Commission on Microbiological Specifications for Foods.

*Commission Regulation (EC) No 2073/2005 **National Conference for Interstate Milk Shipment Pasteurized Milk Ordinance

4.1.2 Characterize the contamination

Companies should characterize the historical contamination of raw materials by analyzing the recording target indicator organism counts or contaminant levels over the last 5 years. For that, companies should estimate the overall lot contamination rate (%) by the following equation:

$$\%\ \text{CONTAMINATION RATE} = \frac{\textit{NUMBER OF ANALYZED SAMPLES}}{\textit{NUMBER OF NON-COMPLIANT SAMPLES}} \times 100$$

Where:

Number of analyzed samples are the total number of raw material samples analyzed that have been tested for microbial indicators or contaminants in a period (5 years).

Number of non-compliant lots are the total number of raw material lots that have exceeded the company or regulatory requirements for a specific microbial indicator (e.g., >10 CFU/g of generic *E. coli*, >1,000 CFU of molds and yeasts) or have surpassed the regulatory limits for contaminants. If for an ingredient, two or more indicators or contaminants are tested, the sum of all tested samples and non-compliant samples for all indicators should be used for that ingredient.

4.1.3 Estimate the annual number of samples

Once the contamination rate has been estimated for each ingredient or raw material, a statistical calculator can be used to estimate the total number of samples to be tested in each ingredient per annual basis. You need to provide the margin of error (usually 5%), confidence level (usually 95%), population size (annual number of lots of each ingredient), and the sample proportion (contamination rate, estimated earlier).

For example, a company that uses 100 lots of specific raw material in a year will need to take 80 samples (one sample from each lot for 80 lots) in order to be representative of the raw material with 5% of margin error, 95% confident, and having 50% of contamination rate.

For an online sample size calculator, go to: **https://select-statistics.co.uk/calculators/sample-size-calculator-population-proportion**

4.1.4 Revise and redesign the sampling plan accordingly

Each year, after testing new samples, the new contamination rate for each ingredient should be calculated by replacing the oldest analyzed samples (oldest year within a 5-year period) by the current year tested samples. For example, on the same 100-lot size example, if year two shows that only 5% of the lots were contaminated, the new sample size would be 43.

4.1.5 Mitigation strategies

Once the company knows the contamination risk of the raw material, it can identify several mitigation strategies to reduce the risk:

a. Create rules for lot acceptance.
Use certain microbial contamination levels of a raw material (e.g., <10 CFU/g) to accept a lot.

b. Classify the suppliers based on risk.
The company could use the microbial verification results of raw materials (contamination rate) and other risk factors to create a scoring system and identify the high-risk suppliers.

Table 5. Risk factors to evaluate the risk of suppliers

Risk factor*	1	3	5	7
Food safety management system	HACCP	Private standard (e.g., ISO)	GMP	None
Implementation	>10 years	5–10 years	<5 years	<1 year
External audits	>5 per year	1–5 per year	1 per year	No audits
Ingredient volume of the total formulation	0.1%	0.1–1%	1–5%	> 5%
Ingredient origin	Local	Regional	National	International
Claims history	No claims	1 claim	1–5 claims	>5 claims
Non-compliant lots with company requirements	<0.1%	<1%	1–5%	>5%

*A score (1, 3, 5, or 7) is assigned to each risk factor. Final risk score is calculated by adding all the scores. The higher the score, the higher the supplier risk.

Reduce or eliminate the high-risk raw materials. Work with the suppliers to reduce the level of contamination by auditing them or including additional risk mitigation measures at the farm or field level, improving the GMPs or GAPs.

CASE STUDY 4

Contamination rate in ground black pepper

Total lots received in 2025: 20 lots

The company tests two microbial indicators for this ingredient:

Generic *E. coli*

Regulatory/company limit:
≤10 CFU/g

Lots tested: 20

Lots >10 CFU/g: 3 non-compliant lots

Molds and yeasts

Regulatory/company limit:
≤1,000 CFU/g

Lots tested: 20

Lots >1,000 CFU/g: 5 non-compliant lots

Because two indicators are tested, you sum across indicators, not across physical lots.

Total tested lots (denominator):
20 lots x 2 indicators = 40 tested results

Total non-compliant lots (numerator):
3 (*E. coli*) + 5 (molds & yeasts) = 8 non-compliant results

Final reported metric	
Number of non-compliant samples	8
Total samples evaluated	40
Non-compliance rate	8 / 40 = 20%

If no historical data exists (e.g., new ingredient or new provider), a default value of 50% contamination rate can be used. If the microbial indicator has never been found in the raw material or ingredient, the company should evaluate the suitability of such indicator in that food matrix.

Activity 3: Design a risk-based sampling plan for microbial indicators in raw materials

1	2	3			4	5	6
Ingredients	Indicators	Evaluate			Technical reasoning	Contamination rate	Total samples per year
		H	E	Q			

1. List all the ingredients you use to make a specific product for which you want to establish a microbial verification plan.
2. List all the potential indicators you may use to assess the quality and safety of each ingredient.
3. Indicate if the indicator is used for evaluating **hygiene (H)**, **environmental (E)**, or **quality (Q)**.
4. Explain the technical reasoning for testing for that indicator. Avoid using "per customer requirement."
5. Estimate the contamination rate for each ingredient. If unknown or never found in the ingredient, use either a 50% default value or use other sources of information *(See Table 4 Sources of information for likelihood and severity)*.
6. Use the **online calculator** to estimate the total annual number of samples to take per ingredient.

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CASE STUDY 5

Example risk-based sampling plan from Activity 3 for microbial indicators identified in flour and milk powder.

List of ingredients	Indicator	Type (quality, environmental, hygiene)	Explanation (Why are we measuring it?)	Samples/ year
 **Flour**	Yeast and mold	Quality	Presence of molds in the flour that could grow in the final product	50
	Generic *E. coli*	Hygiene	Presence of fecal material in the flour; potential presence of pathogenic *E. coli*	
 **Milk powder**	Total Viable Count (TVC)/Aerobic Plate Count (APC)	Quality	Measures the overall microbial load of the ingredient and indicates general hygiene during processing and storage	50
	Enterobacteriaceae	Hygiene	Indicator of post-pasteurization contamination or poor hygienic handling during drying and packaging	40

Activity 4: Design a risk-based sampling plan for pathogens in raw materials

Follow these steps for filling out the calculator on the next page.

1. List all ingredients/final products to establish your risk-based sampling plan for pathogens.
2. Use the activity on identifying hazards (**FDA FSMA Appendix 1, E.U. microbiological criteria**) to identify the pathogens foreseeable to control in the ingredients or final product.
3. Perform a risk assessment of each ingredient based on probability of occurrence and severity. **Assign a score of 1, 3 or 5** for probability (P) and severity (S) for each pathogen and ingredient. Finally, multiply the P x S scores to calculate the final risk for each pathogen.
4. Use the **online calculator** to estimate the total annual number of samples to take per ingredient.
5. Calculate the relative percentage of each pathogen over the total risk.
6. Calculate the number of samples for each pathogen and ingredient by multiplying the relative percentage of each pathogen by the total number of samples.

Activity 4: Calculate the relative risk percentage and total number of samples for each pathogen and ingredient

1	2	3			4	5	6
Ingredient	Pathogen	Probability (P) Low = 1 Med = 3 High =5	Severity (S) Low = 1 Med = 3 High =5	Risk (S x P)	Number of samples per year	Relative risk percentage (%)	Number of samples/ pathogen
				0			
				0			
				0			
			Total Risk	0			
				0			
				0			
				0			
			Total Risk	0			

Reset Form

Automatically calculates for your entered data.

CASE STUDY 6

Example risk assessment and risk-based sampling plan for liquid egg, ground beef, and milk powder as ingredients

Ingredient	Pathogen	Probability (P) Low = 1 Med = 3 High =5	Severity (S) Low = 1 Med = 3 High =5	Risk (P x S)	Number of samples/year	Relative risk percentage (%)	Number of samples/ pathogen
Liquid egg	*Salmonella* spp.	3	5	15	25	83.3%	= 25*0.83 = 21 samples
	E. coli	1	3	3		16.7%	= 25*0.17 = 4 samples
				Total Risk 18			
Ground Beef	*E. coli*	3	3	15	30	75.0%	= 30*0.75 = 22 samples
	Salmonella spp.	1	5	5		25.0%	= 30*0.25 = 8 samples
				Total Risk 20			
Milk powder	*Salmonella* spp.	3	5	15	50	45.4%	= 50*0.45 = 23 samples
	Bacillus cereus	1	3	3		9.1%	= 50*0.09 = 4 samples
	Cronobacter sakazakii	3	5	15		45.4%	= 50*0.45 = 23 samples
				Total Risk 33			

CHAPTER 5

Allergen risk assessment

This section evaluates the potential for allergen cross-contact within the facility and defines controls for validation, monitoring, and verification of allergen sanitation programs. The goal is to prevent undeclared allergens in finished products and ensure compliance with regulatory and customer requirements.

The probability of allergen contamination is assessed based on:
Use of shared equipment or utensils.
Scheduling of allergen and non-allergen products.
Effectiveness of sanitation procedures.
Employee handling and traffic patterns.
Use of rework containing allergens.
Supplier/incoming raw material allergen controls.
Potential for unintended allergen presence in finished products.

Allergen sampling and testing is conducted to:
Confirm incoming raw materials are allergen free.
Validate the effectiveness of an allergen sanitation program.
Verify the performance of ongoing sanitation.
Detect potential cross-contact risks before production.
Assess the risk of unintended allergen presence in finished products.

5.1 Risk-based sampling for allergens

The same principles of risk-based sampling apply to allergens where high-risk areas need to be identified based on the potential risk factors such as:

- **Food contact surfaces** (e.g., mixers, conveyors, fillers) — direct contact with product produces the highest risk.
- **Hard-to-clean areas** (e.g., gaskets, dead ends, valves, hollow rollers) — may harbor allergen residues after sanitation.
- **Changeover complexity** — production lines switching between allergen-containing and allergen-free products.
- **Cleaning effectiveness and frequency** — validated vs. non-validated cleaning procedures.
- **Production scheduling** — sequencing of allergen vs. non-allergen products.
- **Employee practices** — potential for cross-contact via hands, clothing, tools, etc.
- **Adjacent non-food contact surfaces** (e.g., equipment framework, control panels) — medium risk due to indirect transfer.
- **Airflow and dust movement** — especially powdered allergens (e.g., milk powder, flour, peanut dust).
- **Finished product testing** (when applicable) — typically lower risk but used for verification.

How to use these risk factors

- Categorize areas into risk levels (e.g., high, medium, low).
- Determine sampling locations, focusing on worst-case areas.
- Set sampling frequency, where higher risk = more frequent testing.
- Select appropriate test methods (e.g., rapid allergen swabs vs. lab-based methods).

Example

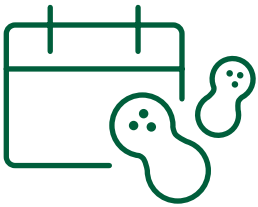
A facility produces both peanut-containing and peanut-free snack bars.

High-risk sampling sites	Medium-risk sampling sites	Low-risk sampling sites
Mixer and conveyor after peanut product runs.	Equipment framework near production line.	Finished peanut-free product. (For verification only)
Filler nozzles and product containing surfaces.	Utensils and employee gloves.	

Sampling plan outcome (from example on previous page)

- Swab high-risk areas after every allergen changeover.
- Swab medium-risk areas daily or weekly.

Test finished product periodically to verify overall control.



Timing for sampling

Timing for sampling is typically pre-operational (i.e., after cleaning, before production), for post-sanitation validation studies, and for routine verification per risk-based schedule.

For example, in a facility that manufacturers both allergen-containing and non-allergen-containing products, sampling timing may be adjusted based on identified risk factors such as changeover complexity, cleaning effectiveness, and equipment design.

Timing for sampling	
After production of an allergen-containing product	Swab high-risk food contact surfaces (e.g., mixers, conveyors, fillers) immediately following cleaning to verify allergen removal.
Before the next non-allergen product run (pre-operational)	Conduct targeted swabbing of hard-to-clean areas (e.g., gaskets, valves) and adjacent non-food contact surfaces.
During initial runs of non-allergen products (e.g., first 1–2 production runs after changeover)	Perform additional verification sampling or finished product testing to confirm controls are effective.
After full sanitation between allergen and non-allergen runs	Implement intensified sampling across high- and medium-risk areas to validate the effectiveness of the cleaning event.

Activity 5: Design a risk-based sampling plan for potential allergens

Identify a production line and classify risk of potential allergen contamination in at least 10 locations on that line.

1	2	3			4	5
Location	Allergen	Risk level			Justification for level of risk	Timing of sampling
		Low	Med	High		

1. List locations at risk of potential allergen contamination.
2. List all the potential allergen sources for this line.
3. Indicate the level of risk for allergen contamination.
4. Explain why this location is assigned this risk level.
5. List how often this location should be sampled.

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5.2 Validation of allergen control (quantitative methods)

Validation of allergen sanitation protocols demonstrates that cleaning removes allergens to acceptable levels. *See FAO/WHO recommended allergen thresholds.*

A correct validation experiment must be conducted with these key elements:

- Use of quantitative tests (e.g., ELISA measuring the concentration of the allergen in ppm).
- Target the specific allergen to validate (e.g., validation of allergen sanitation for milk residues).
- Use detection limits meeting regulatory and customer requirements (e.g., the recommended threshold for milk is 2 ppm).
- Be performed under worst-case conditions (e.g., hard to clean areas, spots with historical noncompliance).

The experimental design for the validation of an allergen sanitation program should include:

- Collect a minimum of five surface samples per equipment before and after cleaning from different areas of the equipment. **Special attention should be given to hard-to-clean areas.**
- Collect the surface samples during at least three sanitation cycles.
- All post-cleaning results from all samples must be under the allergen detection limit or action threshold.
- If validation fails (e.g., the surface samples contain allergen concentration surpassing the threshold), change the sanitation program and re-validate the sanitation program.

After successful validation, ongoing verification is sufficient unless new allergens, equipment, chemicals, or major process/formulation changes occur.

CASE STUDY 7

Validation of sanitation for milk allergen on a cookie production line

A cookie manufacturer produces two products on the same line:

Cheese-filled cookies containing milk allergen

Plain sugar cookies with no milk ingredient

Because both products run on shared equipment, the company must validate that the sanitation program effectively removes milk residues before producing the non-milk cookies.

Acceptance criterion

All post-cleaning surface samples must be below the detection limit or action threshold: **<2.0 ppm milk protein.**

Worst-case conditions selected for validation

- Production of the cheese-filled cookie, which has the highest milk content on the line.
- End of a long production run, when residues are more built up.
- Sampling of hard-to-clean areas, such as:
  - Depositor nozzles
  - Scraper blades
  - Conveyor belt joints
  - Hopper corners
  - Wire-cut assembly
  - Gaskets and seals
  - Transfer chutes

Validation study design

Parameter	Description
Product used for validation	Cheese-filled cookies containing milk (worst-case product)
Allergen tested	Milk protein
Analytical method	Quantitative ELISA
Detection limit	1.0 ppm
Action threshold	2.0 ppm
Number of sanitation cycles	3
Samples per cycle	At least five food contact surface samples from different equipment areas
Focus areas	Hard-to-clean zones (nozzles, joints, gaskets, blades)
Validation criteria	All post-cleaning samples must be <2 ppm

CASE STUDY 7 continued

Validation results

Equipment/ area	Sample type	Cycle (ppm milk protein)			Acceptance criteria
		1	2	3	
Hopper interior	Pre-cleaning	185	172	201	N/A
Depositor nozzle	Pre-cleaning	240	198	215	N/A
Wire-cut blade	Pre-cleaning	96	88	102	N/A
Conveyor belt joint	Pre-cleaning	58	63	71	N/A
Scraper blade	Pre-cleaning	122	110	134	N/A
Hopper interior	Post-cleaning	<1.0	<1.0	<1.0	Pass
Depositor nozzle	Post-cleaning	<1.0	<1.0	2.8	Fail in cycle 3
Wire-cut blade	Post-cleaning	1.3	<1.0	<1.0	Pass
Conveyor belt joint	Post-cleaning	<1.0	1.5	<1.0	Pass
Scraper blade	Post-cleaning	<1.0	<1.0	<1.0	Pass
Transfer chute	Post-cleaning	<1.0	<1.0	<1.0	Pass
Gasket/seal area	Post-cleaning	1.1	<1.0	<1.0	Pass

This **cycle fails**, because one post-cleaning sample exceeds the 2 ppm threshold. The result of 2.8 ppm at the depositor nozzle shows that the sanitation procedure is not consistently effective under worst-case conditions. That means the sanitation program is **not yet validated**.

CASE STUDY 7 continued

Corrective action

The company reviews the cleaning procedure and identifies that the depositor nozzle has internal dead spots and is not being fully disassembled during routine sanitation.

They revise the sanitation program by:

- Requiring full nozzle disassembly.
- Adding a dedicated brushing step.
- Increasing detergent contact time.
- Retraining sanitation staff.

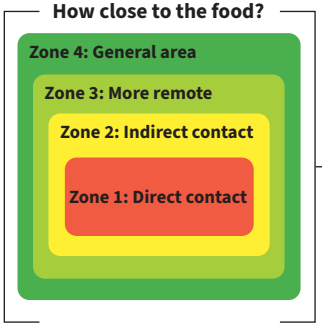
Re-validation

The revised sanitation procedure is then re-validated over three new sanitation cycles using the same worst-case product, same sampling locations, and same quantitative milk ELISA method. If all post-cleaning samples from all three cycles are below **2 ppm**, the sanitation program is considered **validated**.

5.3 Verification (qualitative methods)

Verification ensures sanitation controls work over time by using qualitative lateral flow allergen swabs (presence/absence) on high-risk food contact surfaces after cleaning but before production. Test high-risk zones (Zone 1) after each changeover or weekly, medium-risk zones (Zone 2) monthly, and low-risk zones (Zones 3 and 4) quarterly or annually. Positive tests or customer complaints require immediate re-cleaning, re-testing, root cause analysis, corrective action, and possible retraining. Review allergen verifications annually, after process or allergen changes, or following any allergen-related incident.

Figure 1: Identification of high-risk sampling sites



Zone	How hard is it to clean?	Low	Medium	High
	High (Typically Zone 1)	Mid-level priority	High priority	High priority
	Medium (Typically Zone 2 and 3)	Low priority	Mid-level priority	High priority
	Low (Typically Zone 4)	Low priority	Low priority	Mid-level priority

Allergen testing focused on direct contact surfaces; Zones 1 and 2 (swabs or CIP).

Hard to clean and direct contact should be tested after every change over.

- High frequency testing
- Once per week/month
- Low frequency testing

5.4 Allergen calculation examples

5.4.1 Example 1: Converting gliadin to gluten

If an allergen such as gluten is analyzed and the test kit determines gliadin, we need to know the proportion that this protein represents in the food in order to express the result as gluten, which is the allergen referenced in the reference dose (Rfd) values. If gliadin represents half of the gluten proteins in the analytical report, the result must be multiplied by 100/50 = a factor of 2.

5.4.2 Example 2: Converting to allergenic protein

Calculations to be performed when the result is expressed in mg/kg of the allergenic ingredient rather than in mg/kg of allergenic protein per kg of food.

Hazelnut

We have a kit that reports the result in mg/kg of hazelnut, and based on the calibration curve used, we know that these hazelnut concentrations contain 15% protein. Therefore, it is necessary to multiply the reported result (mg/kg of hazelnut) by 0.15 to express it as mg of hazelnut protein per kg of food.

Reported result: 15 mg/kg of hazelnut

15 mg/kg x 0.15 = 2.25 mg/kg of hazelnut protein

Reference dose (RfD): The reference dose is 3 mg of allergenic protein per kg of food.

AL (Action Level): For 100 g (0.1 kg) of hazelnut (RfA): RfD / RfA = AL

3 mg/0.1 kg = 30 mg/kg of hazelnut protein = AL

Since the obtained result, expressed as protein per kg of food, is 2.5 mg of protein/kg, labeling would **not** be required.

Egg

We have a test kit that reports the result in mg/kg of whole egg powder. Based on the information provided in the kit insert, we know that a factor of 0.48 must be applied to convert the result to total egg protein per kg of food. Therefore, it is necessary to multiply the result reported by the kit by this factor.

Reported result: 10 mg/kg of whole egg powder

10 mg/kg x 0.48 = 4.8 mg/kg of total egg protein

If we want to convert the result specifically to egg white protein, it is necessary to apply a factor of 0.263.

Reported result: 10 mg/kg of whole egg powder

10 mg/kg x 0.263 = 2.63 mg/kg of egg white protein

Using these data, it would also be possible to convert a result expressed in mg of egg white protein per kg of food into mg of total egg protein per kg of food.

RfD: The reference dose for this allergenic ingredient is 2 mg of total egg protein per kg of food.

AL: We calculate the action level for 100 g (0.1 kg) of total egg protein (RfA): RfD/RfA = AL

2 mg/0.1 kg = 20 mg/kg of total egg protein

Since the obtained result, expressed as total egg protein per kg of food, is 4.8 mg of protein/kg, labeling would **not** be required.

The issue would arise if the kit in question did not indicate the conversion factor (i.e., the percentage of protein present in the allergenic ingredient used in the calibration curve).

CHAPTER 6

Mycotoxin risk assessment

6.1 Risk-based sampling of mycotoxins

This risk assessment evaluates the potential for mycotoxin contamination within raw materials, in-process products, and finished goods. It defines controls for sampling, validation, monitoring, and verification to ensure mycotoxin levels remain within regulatory and customer-defined limits.

Mycotoxin contamination can occur for many reasons, including biological (susceptibility of the crop), environmental (temperature, moisture, till practices, and crop rotation), harvesting (crop maturity), and storage (temperature, moisture, duration, and cleanliness).

6.1.1 How fine must a sample be ground before testing, and why is it important?

Mycotoxins are unevenly distributed in grain, making detection difficult—a single corn kernel can contain high toxin levels. Concentrations are measured in ppm (1 mg/kg) and ppb (1 µg/kg); 1 ppm equates to about 4–5 contaminated kernels per semi-truck, while 1 ppb means one affected kernel among hundreds of truckloads. Because these contaminants are rare and inconsistent, representative sampling is crucial. Federal Grain Inspection Service (FGIS) guidelines require specific probe patterns for trucks and rail cars — five probes for flat bottoms, two for hopper bottoms. Samples should be ground so that 95% passes through a 20-mesh screen, ensuring accurate tests; uneven grinding produces unreliable results. Equipment must be cleaned between samples to avoid cross-contamination.

6.1.2 Validation (quantitative methods)

Validation shows that preventive controls — like supplier approval, CoAs, segregation, or processing steps — effectively reduce mycotoxin risks to acceptable levels. This process uses quantitative methods (e.g., LC-MS/MS, HPLC, ELISA, or LFD) that must meet criteria for detection limits, accuracy, precision, and recovery.

Validation follows Codex Alimentarius and AOAC INTERNATIONAL guidelines, typically using 10–30 samples per lot across multiple lots or seasons, with replicate tests confirming reliability. Validation is required for new suppliers, sources, commodities, mycotoxins, or major process changes, and results must be below regulatory or internal limits.

6.1.3 Verification (qualitative and quantitative methods)

Verification ensures that mycotoxin controls remain effective over time, using independent lab tests on products, rapid screening like LFDs, and periodic quantitative confirmation. Exceeding limits leads to corrective actions such as lot rejection, supplier action requests, root cause analysis, or increased sampling.

Verification frequency is based on commodity risk. Commodities should be evaluated using defined risk factors to determine whether they are high, medium, or low risk for mycotoxin contamination.

Commodity risk should be assessed based on factors such as:

- **Inherent susceptibility of the commodity:**
  - High risk: corn, peanuts, tree nuts, wheat.
- **Geographic origin and climate conditions:**
  - Warm, humid regions increase fungal growth and mycotoxin production.
- **Pre-harvest conditions:**
  - Drought stress, insect damage, and plant disease increase risk.
- **Post-harvest handling and storage:**
  - Moisture control, drying practices, and storage conditions (e.g., temperature, humidity).
- **Supplier controls and history:**
  - Preventative programs, testing data, audit results, past non-conformance.
- **Historical data and trends:**
  - Known contamination patterns for specific commodities and regions.
- **Supply-chain complexities:**
  - Multiple handlers, storage steps, or repacking increases variability.

Based on this evaluation:

High-risk commodities are verified per lot or shipment.

Medium-risk commodities are verified monthly or by supplier

Low-risk commodities are verified quarterly or annually.

Frequency may increase due to positive results, seasonal risks, adverse weather conditions, non-conformance, or regulatory/customer requirements.

Learn more about mycotoxin testing solutions

Connect with a Food Safety expert

CASE STUDY 8

Validation of sanitation for aflatoxin sampling plan for imported maize using the FAO Sampling Tool

Background

A maize milling company imports bulk shipments of maize grain from multiple suppliers. Because maize is susceptible to contamination by *Aspergillus* species producing aflatoxins, the company must verify that each shipment complies with the applicable regulatory limits before the grain is used in food production.

The company's quality assurance team wants to ensure that its sampling plan is robust and risk-based, meaning that it effectively distinguishes compliant lots from contaminated ones while minimizing unnecessary rejection of compliant shipments.

To evaluate possible sampling strategies, the team uses the FAO Mycotoxin Sampling Tool, which allows comparison of sampling plans and estimation of the probability of accepting or rejecting lots at different contamination levels.

Regulatory context

For this case study, the following regulatory limit is used:

Parameter	Value
Commodity	Maize
Mycotoxin	Total aflatoxins
Regulatory limit	20 µg/kg (ppb)

This limit represents the **maximum** concentration allowed for the commodity.

Objective of the sampling plan

The objective of the sampling plan is to:

1. **Detect lots that exceed 20 ppb aflatoxin.**
2. **Minimize the probability of accepting contaminated lots *(buyer's risk)*.**
3. **Avoid unnecessary rejection of compliant lots *(seller's risk)*.**
4. **Identify whether improvements in sampling intensity significantly improve decision reliability.**

Link to download the tool:
https://tools.fstools.org/mycotoxins/

See Annex 2 for the steps to use the FAO Mycotoxin Sampling Tool.

CASE STUDY 8 continued

Determine sampling strategy

Step 1: Define common parameters in the FAO tool

The quality assurance team opens the Edit Plans tab in the FAO sampling tool and enters the common parameters.

Parameter	Value used
Mycotoxin/commodity	Aflatoxin/shelled corn
Kernel count per kg	Default value from the tool
Regulatory limit	20 ng/g
Analytical variance	Within laboratory

These parameters define the regulatory threshold and analytical assumptions used to calculate the operating characteristic (OC) curves.

Step 2: Define sampling plans to compare

The team decides to compare **three** possible sampling strategies to understand how sampling intensity affects reliability.

Sampling plan	Laboratory sample size	Test portion	Number of aliquots	Accept/reject limit
Plan A (minimal)	5 kg	25 g	1	20 ppb
Plan B (moderate)	10 kg	50 g	1	20 ppb
Plan C (robust)	10 kg	100 g	2	18 ppb

Rationale for the plans

Plan A (minimal)

Represents a basic sampling program with relatively small laboratory samples.

Plan B (moderate)

Increases laboratory sample size and analytical test portion.

Plan C (robust)

Further improves reliability by:

- Increasing analytical test portion.
- Using replicate aliquots.
- Using a slightly lower accept/reject threshold.

CASE STUDY 8 continued

Step 3: Evaluate variance contributions

After saving the plans, the team opens the **Chart Results** tab and reviews the variance analysis.

The tool separates total variability into three components:

Variance source		Interpretation
1	Sampling variance	Variation caused by heterogeneous contamination in the lot
2	Sample preparation variance	Variation during grinding and sub sampling
3	Analytical variance	Variation during laboratory analysis

Example interpretation

The results show that **sampling variance** accounts for the largest portion of total variability.

This indicates that improving reliability requires increasing **sample size**, rather than only improving analytical precision.

This finding is consistent with mycotoxin research showing that **sampling error** dominates *total testing uncertainty*.

Step 4: Analyze the operating characteristic (OC) curves

The OC curve shows the probability that a lot will be accepted depending on its true aflatoxin concentration.

Example interpretation of results

True aflatoxin level	Plan A acceptance probability	Plan B acceptance probability	Plan C acceptance probability
10 ppb	98%	99%	99%
20 ppb	50%	50%	45%
25 ppb	60%	40%	25%
30 ppb	35%	20%	10%

CASE STUDY 8 continued

Interpretation of buyer's risk

Buyer's risk occurs when a contaminated lot is incorrectly accepted.

Example: If the *true aflatoxin level is 25 ppb*, the lot exceeds the regulatory limit.

	Probability contaminated lot is accepted
Plan A	60%
Plan B	40%
Plan C	25%

This means the minimal sampling plan (Plan A) would allow **more than half of contaminated lots to pass inspection.**

Increasing sample size significantly reduces this risk.

Interpretation of seller's risk

Seller's risk occurs when a compliant lot is rejected.

Example: If the *true aflatoxin level is 15 ppb*, the lot is compliant.

	Probability lot is rejected
Plan A	12%
Plan B	8%
Plan C	10%

This illustrates the typical tradeoff:

- Stronger sampling plans reduce buyer's risk.
- But may slightly increase seller's risk.

Final decision

Based on the FAO tool results, the company decides to implement *Plan B*.

Justification

Plan B provides a good balance between reliability and practicality:

- Buyer's risk is significantly reduced compared with Plan A.
- Seller's risk remains acceptable.
- Sampling effort is operationally feasible.

CASE STUDY 8 continued

Final sampling protocol implemented

Step		Procedure
1	Lot identification	Identify incoming maize shipment
2	Increment collection	Collect multiple increments across the lot
3	Composite sample	Combine increments to create a 10 kg laboratory sample
4	Sample preparation	Grind the full laboratory sample
5	Test portion	Extract 50 g for aflatoxin analysis
6	Analysis	ELISA or HPLC quantification
7	Decision	Accept if ≤20 ppb; reject if >20 ppb

Key lessons from the case study

Lesson	Explanation
Sampling dominates uncertainty	Most variability comes from heterogeneous contamination
Larger samples improve reliability	Increasing laboratory sample size significantly reduces risk
OC curves are decision tools	They show the probability of correct or incorrect classification
Risk-based sampling improves inspection programs	Tools like FAO allow objective comparison of sampling plans

CHAPTER 7

Risk-based environmental monitoring program (EMP)

7.1 Risk-based environmental monitoring program (EMP)

Sampling and testing of the environment and equipment within a food manufacturing facility to prevent cross-contact contamination of the product with the environment.

Whether it is validating the hygienic design of equipment or verification of specific pre-requisite programs (e.g., sanitation and sanitary equipment design), a risk-based approach to EMP must be logical and consistent with other aspects of food safety and quality management, such as HACCP.

7.1.1 The reality: EMPs are not truly risk-based

Companies have EMP programs implemented but don't have the risk assessment principles:

- Too many sites with no risk prioritization.
- Historical site selection ("We've always swabbed there," with no documented rationale).
- Wrong tests.
- Data with no decisions.

If food safety systems are risk-based, environmental monitoring must be risk-based.

A risk-based EMP program should include:

- List of well-defined risk factors that affect environmental contamination.
- Risk-prioritized list of surfaces and equipment.
- Allocation of sampling resources (type and frequency) based on risk level.

Dos and don’ts in a EMP

- Zone 1 samples should be tested for indicator organisms like coliforms, *Enterobacteriaceae*, or total plate counts.
- If testing for pathogens in Zone 1, a positive result may require holding all products produced on that line from one cleanup to the next.
- The company must have a documented policy for managing products while awaiting Zone 1 results.
- Collect samples after cleanup but before sanitizers, and also during operations if needed.
- Neutralize any residual sanitizer in pre-operational samples.
- When sampling during production, establish baseline microbial loads, as sampling is less useful without this data.
- Zone 4 samples should be collected to check for potential pathogen harborage sites that might access production areas.
- This sampling also evaluates the effectiveness of barriers preventing pathogens from moving into Zone 3.
- Prioritizing areas that connect to the plant or where foot or vehicle traffic could introduce contamination.

Table 1. Example of recommended microbiological indicator limits for equipment cleaning before and after application of sanitizer provided by Almond Board of California

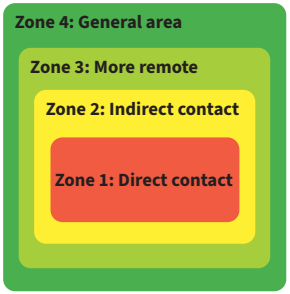
Quantitative microbiological indicator test	Target/ acceptable limits	Post-heat treatment taken before sanitizer (CFU/40 in² [250 cm²])	Post-heat treatment — pre-op taken after sanitizer (CFU/40 in² [250 cm²])
Aerobic Plate Count	Target	<100	<10
	Acceptable	<500	<100
Coliforms	Target	<10	<10
	Acceptable	<100	<50
Total *Enterobacteriaceae*	Target	<10	<10
	Acceptable	<100	<50

7.1.2 Basics of a risk-based environmental program

Zoning

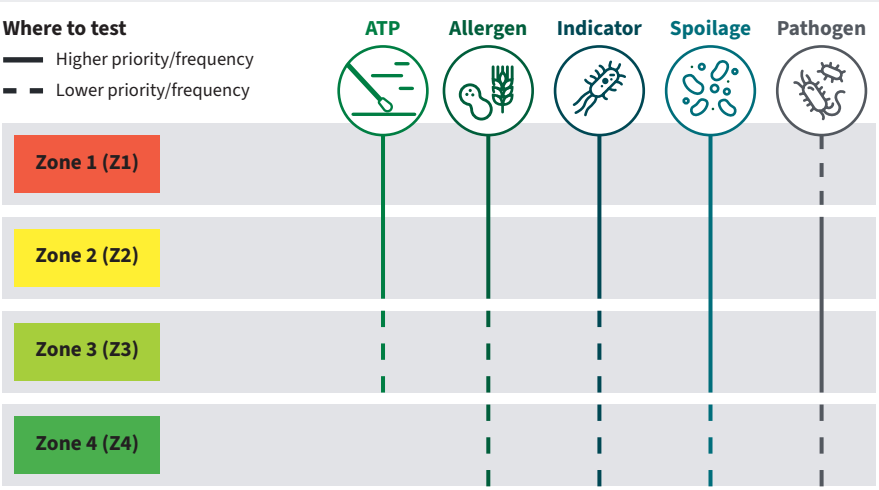
As you begin the planning process, you first need to identify the sampling zones in the production environment. This is not to be confused with hygienic zoning, which is segregation of areas or rooms in the plant. The definition of each zone is outlined here.

Figure 1. Zoning map



Zone 1	Surfaces directly in contact of the food or in which the food is exposed.
Zone 2	Non-food contact surfaces and areas adjacent to Zone 1, such as control panels and equipment frames.
Zone 3	Non-food contact surfaces located in or near processing areas, such as floors, walls, drains, ceilings, etc.
Zone 4	Areas outside the production area.

Figure 2. Type of test depending on the zone



EMP should be approached holistically, encompassing a range of tests to ensure both food safety and quality. Figure 2 shows zones that are typically sampled for ATP, Indicators, pathogens, and allergens.

7.1.3 Design of a risk-based environmental program

The design of a risk-based EMP should include the following steps:

Steps to design a risk-based EMP

1. Identify sampling sites and zoning

a. List all equipment and surfaces after the last CCP (e.g., cooking, disinfection).

b. Identify the zone (1, 2, 3 and 4).

2 . Define the risk factors and numerical scoring system

Question 1: Proximity to product (How close is this surface to exposed product or post-lethality handling?)

1 = Far from product/before kill step.

2 = Near product but with controls in place.

3 = Directly near exposed or post-lethality product.

Question 2: Hygiene zone (Based on the process flow, which hygiene zone does this surface belong to?)

1 = Zone 4.

2 = Zone 3.

3 = Zones 1–2.

Question 3: Surface design & cleanability (How do the design, material, and accessibility of this surface influence effective cleaning and control?)

1 = Smooth, accessible, easy to clean and inspect.

2 = Some design limitations or partial access challenges.

3 = Complex design, damaged surfaces, or harborage-prone, and difficult to clean consistently.

Question 4: Moisture & residue (Does this area tend to remain wet or accumulate product or organic residue?)

1 = Typically dry and clean.

2 = Occasionally wet or with residue.

3 = Frequently wet or with accumulation.

Question 5: Traffic & handling (How often does this surface come into contact with people, tools, or movement?)

1 = Infrequent contact.

2 = Occasional contact.

3 = Frequent or constant contact.

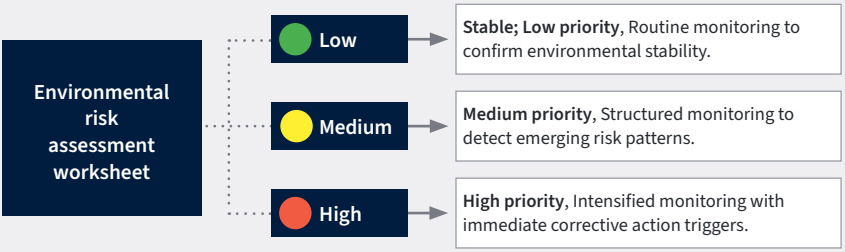
Question 6: Historical performance & recurrence (Has this surface or area shown historical contamination events, repeated findings, or control challenges?)

1 = No known history of findings or recurring issues.

2 = Occasional findings or isolated events.

3 = Repeated findings, trends, or documented control challenges.

Figure 3: Risk levels in a risk-based EMP



3. Prioritize high-risk areas

Estimate the final score of each site/surface/equipment evaluated by adding the individual scores of each question. Establish a high, medium and low scale.

For example: There are six questions and each question can have a 1–3 score, so the minimum score is six and the maximum score is 18. Taking even levels, low risk can go from six to nine, medium risk from 10 to 13, and high risk from 14 to 18.

4. Assign frequencies to risk level

Each risk level should have a frequency of testing. The higher the risk, the higher the frequency.

How many samples?

The number of samples taken on each monitoring day should be based on the size and complexity of the facility, in addition to the practicality of implementing the program. The frequency of sampling should be evaluated in accordance with the relative risk of a quality failure, should pre-established cut-off levels be exceeded. Facilities in which environmental monitoring results frequently reveal poor sanitation or emerging microbial harborage sites should increase the frequency of sampling. The same risk evaluation should be used to determine how frequently results should be evaluated by a food safety and quality team.

Figure 4: Advantages of a risk-based EMP

The scoring criteria, and risk thresholds are intended as a generic framework and that each facility should customize them based on its products, processes, equipment, facility layout, historical findings, and applicable regulatory/customer requirements.

CASE STUDY 9

Risk-based EMP for a RTE meat products

A food manufacturing facility produces ready-to-eat (RTE) sliced deli meat that undergoes a lethality treatment (thermal processing) before entering a post-lethality slicing and packaging area.

After lethality, the product is exposed to the environment before packaging, making this area highly sensitive to contamination. Because microbial contamination can occur through contact with equipment or environmental surfaces, the facility needs to design an EMP to verify sanitation effectiveness and detect potential contamination sources.

The post-lethality environment is characterized by:

- Wet cleaning sanitation procedures.
- Frequent employee movement between equipment.
- Multiple food contact and non-food contact surfaces.

Objective of the EMP

The EMP aims to:

1. Verify that sanitation procedures effectively control microbial contamination.
2. Detect the presence of pathogens or indicator organisms in the environment.
3. Identify potential harborage sites for contamination.
4. Prevent contamination of RTE products before packaging.

Description of the post-lethality environment

Parameter	Description
Process	Slicing and packaging of cooked deli meat
Product exposure	Product exposed to environment before packaging
Cleaning method	Wet sanitation
Employee movement	Operators move between slicers and conveyors
Food safety concern	Post-lethality contamination with *Listeria monocytogenes*

CASE STUDY 9 continued

EMP sampling locations

The facility identifies several environmental sampling sites that represent different contamination risks.

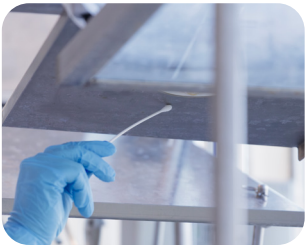
Figure 5. EMP sampling locations in RTE meat facility

ZONE 1

Food Contact Surface

Sampling site:
Slicer frame near product discharge

Rationale:
Direct contact with product after lethality



ZONE 2

Non-Food Contact Near Product

Sampling site:
Conveyor underside after lethality

Rationale:
Potential contamination source from equipment

ZONE 3

Non-Food Contact Environmental Site

Sampling site:
Floor drain near packaging line

Rationale:
Possible harborage site for pathogens

CASE STUDY 9 continued

Example risk-based EMP for a RTE meat facility

Sample area/ surface	Proximity to exposed product	Hygiene zone	Surface design & cleanability	Moisture/residue potential	Traffic & handling	Historical performance & recurrence	Overall risk	Observations/ rationale: What type of testing and why
Slicer frame near product discharge	3	3	2	3	3	1	High	ATP, TPC after each use, Add LAB, EB
Floor drain near the packaging line	2	2	3	3	2	2	Med	*Listeria* spp., *Salmonella*, EC
Packaging line control panel	1	2	1	1	3	2	Med	EB, Air ET
Changeover tools stored between runs	3	3	1	1	3	2	Med	PEM Protein swab after cleaning, validation, specific allergen test between allergen containing products

Low Risk Score 6–9	**Stable; Low priority**, Routine monitoring to confirm environmental stability.
Med Risk Score 10–13	**Medium priority**, Structured monitoring to detect emerging risk patterns.
High Risk Score 14–18	**High priority**, Intensified monitoring with immediate corrective action triggers.

Activity 6: Environmental risk assessment worksheet

This worksheet is a discussion and prioritization tool to support risk-based environmental monitoring decisions, not an audit or compliance checklist.

1	2						3	4
Sample area/ surface	**Proximity to exposed product** How close is this surface to exposed product or post-lethality handling?	**Hygiene zone** Based on the process flow, which hygiene zone does this surface belong to?	**Surface design & cleanability** How do the design, material, and accessibility of this surface influence effective cleaning and control?	**Moisture/ residue potential** Does this area tend to remain wet or accumulate product or organic residue?	**Traffic & handling** How often does this surface come into contact with people, tools, or movement?	**Historical performance & recurrence** Has this surface or area shown historical contamination events, repeated findings, or control challenges?	**Overall risk**	**Observations/ rationale** Please include what type of testing you would include and why

1. Identify the surface or sampling location.
2. Assess each risk factor using the guidance provided (page 67).
3. Calculate the overall risk score.
4. Document the rationale for the assessment.
5. Use the risk level to define the monitoring strategy (priority, frequency, methods, etc.).

Reset Form

Low Risk Score 6–9	**Stable; Low priority**, Routine monitoring to confirm environmental stability.
Med Risk Score 10–13	**Medium priority**, Structured monitoring to detect emerging risk patterns.
High Risk Score 14–18	**High priority**, Intensified monitoring with immediate corrective action triggers.

5

7.1.4 Risk management: Seek and destroy

It is paramount to take a multi-faceted systematic approach to finding sites of persistent strains (niches) in food processing plants, with the goal of either eradicating or mitigating effects of these strains. This process has been used effectively to address persistent *Listeria monocytogenes* contamination in food processing plants.

Companies must establish limits for indicator organisms and define corrective actions for when results exceed these limits or when pathogens are detected. For Zone 1 positives, corrective actions may include the following steps (*Stier, 2026*):

1	Placing product from the affected line on hold.
2	Quarantining the suspect area and limiting access to that area.
3	Disassembling and inspecting equipment, followed by additional vector sampling.
4	Initiating additional cleaning and sanitation activities.
5	Conducting additional sampling in Zones 1, 2, and 3 around the area of the initial positive result prior to cleaning. (The goal should be three straight days of negative tests before returning to normal adherence to the EMP program.)

Learn more about environmental monitoring

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CHAPTER 8

Cost of poor quality

8.1 Building a quality toolbox

Sanitation and quality programs are often seen as a business expense that in times of budget constraints are massively cut. This can have unintended consequences for the company, leading to food quality and sanitation failures that lead to profitability losses and customer lack of confidence.

Estimating an adequate quality control program budget is crucial for avoiding future quality failures and business profit losses. Quality control program (QCP) budget may include:

Acceptable failure rate (%) that the QCP will be based on.
Labor, testing.
Evaluation (appraisal) of GMP, HACCP, EMP.
Costs of dealing with expected routine internal failures (e.g., hold and release, rework, disposition, etc.).
Programs used to manage external failures such as customer complaints, regulatory management.
Continuous improvement based on deviations from failure rate and failures.

Many quality departments use some measure of quality cost/revenue or cost dollars/profit dollars to track the performance of the QCP the business. Depending on your company, you may have a target COQ of, say, 15% of sales revenue.

A higher percentage of product returns due to quality failures can be costly for food companies. Companies should have mechanisms to estimate the costs of a particular failure that could include:

- Original cost to make the product (e.g., ingredients and materials, labor, overhead, storage fees, etc.).
- Costs of inspecting to separate potentially good from bad product.
- Costs to destroy or reprocess the poor product.
- New ingredients and materials and any labor to make the product again.

Microbial indicators are also very useful to identify potential food quality and sanitation.

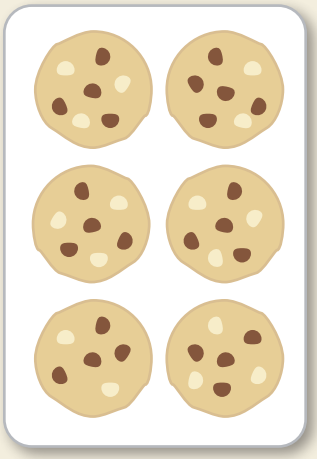
CASE STUDY 10

Cost of poor quality in a cookie manufacturing facility

Background

A medium-size cookie manufacturer produces 30,000 kg of cookies per week for retail distribution. The company operates under a quality control program that includes:

- GMP verification.
- HACCP monitoring.
- Environmental monitoring program (EMP).
- Routine microbiological testing.
- Finished product quality testing.



During a period of budget constraints, management decided to reduce the sanitation and quality control budget by 40%. The following changes were implemented:

- Reduced environmental monitoring samples.
- Reduced finished product testing.
- Reduced sanitation verification checks.
- Eliminated some internal quality audits.

Six months after these changes, the company experienced a significant increase in product quality complaints and internal product failures.

Production and economic context	
Parameter	**Value**
Weekly production	30,000 kg cookies
Production cost	$2.20/kg
Selling price	$3.50/kg
Weekly revenue	$105,000
Weekly production cost	$66,000
Weekly gross margin	$39,000

CASE STUDY 10 continued

Quality control program budget
Before budget reduction, the company invested approximately 15% of sales revenue in quality programs, which included sanitation verification and testing.

QCP component	Annual cost
Labor (QA technicians)	$220,000
Microbiological testing	$85,000
Environmental monitoring	$60,000
Internal audits and verification	$40,000
Complaint handling and investigations	$45,000
Continuous improvement activities	$50,000
Total QCP cost	**$500,000**

This represented approximately **14–15% of annual sales**, which was consistent with company targets. After the budget cuts, the QCP cost was **reduced to $300,000 per year**.

Expected failure rate
Before the budget cuts, the company's acceptable failure rate was:

Failure type	Acceptable rate
Internal product failures	1%
Customer complaints	0.2%
Product returns	0.1%

These rates were historically stable.

Quality failure event
Three months after the budget cuts, microbial indicators from finished product testing showed **elevated mold counts in chocolate chip cookies.**

The problem was traced to **insufficient sanitation** of the cooling conveyor belt, which allowed mold spores to accumulate and contaminate finished cookies.

Because environmental monitoring had been reduced, the issue was **not detected** early.

CASE STUDY 10 continued

Product recall scenario
The contamination led to the following event:

Parameter	Value
Production affected	12,000 kg cookies
Distribution status	Already shipped to retailers
Reason for recall	Elevated mold levels

Cost of the quality failure

Original production cost	
Ingredients and packaging	$18,000
Labor	$5,500
Overhead	$3,500
Storage and logistics	$2,000
Total production cost	**$29,000**

Recall and inspection costs	
Product retrieval from retailers	$7,000
Inspection and segregation	$4,000
QA investigation	$2,500
Total recall management cost	**$13,500**

Because mold contamination could not be removed, the entire batch was destroyed.

Product disposition	
Destruction of product	$3,500
Waste disposal	$1,500
Total destruction cost	**$5,000**

To fulfill retailer demand, the product had to be produced again.

Reproduction cost	
New ingredients	$18,000
Labor	$5,500
Overhead	$3,500
Total production cost	**$27,000**

CASE STUDY 10 continued

Total cost of the failure	
Production cost of lost product	$29,000
Recall and inspection	$13,500
Product destruction	$5,000
Reproduction cost	$27,000
Total failure cost	**$74,500**

Impact on profitability	
Weekly gross margin	$39,000
Failure cost	**$74,500**

Root cause analysis
Investigation identified the following contributing factors:

Cause	Explanation
Reduced sanitation verification	Conveyor sanitation was not routinely verified
Reduced EMP sampling	Mold growth on equipment went undetected
Fewer QA inspections	Early warning signals were missed
Budget reduction	Quality monitoring capacity was reduced

Role of microbial indicators
Routine microbial indicators such as mold and yeast counts are essential to detect sanitation failures. Example monitoring data:

Sample location	Mold count before event	Mold count during event
Cooling conveyor	<10 CFU/swab	120 CFU/swab
Packaging area	<5 CFU/swab	15 CFU/swab
Finished cookies	<10 CFU/g	85 CFU/g

These results indicated **sanitation failure and environmental contamination.**

CASE STUDY 10 continued

Corrective actions

The company implemented the following improvements:

1. **Restore environmental monitoring program**
   Detect contamination earlier
2. **Increase sanitation verification**
   Prevent equipment contamination
3. **Re-establish internal audits**
   Identify process deviations
4. **Implement trend analysis of microbial indicators**
   Early detection of sanitation failures

Lessons learned

Lesson	Explanation
Quality programs protect profitability	Preventive programs are cheaper than failures
Sanitation failures can quickly become expensive	One event can erase weeks of profit
Microbial indicators provide early warning	Reduced testing increases risk
Cost of poor quality is often underestimated	Failures include hidden operational costs

Key conclusion

Reducing investment in sanitation and quality programs can create hidden operational risks that ultimately lead to higher costs than the savings generated by budget cuts.

Preventive quality control programs are therefore a strategic investment rather than a business expense.

References

1. Bassett J, Jackson T, Jewell K, Jongenburger I, Zwietering MH. Impact of microbial distributions on food safety. Brussels (Belgium): ILSI Europe; 2010. (ILSI Europe Report Series). ISBN 978-9078637202. **https://ilsi.eu/publication/impact-of-microbial-distributions-on-food-safety/**

2. European Commission. (2005). *Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs*. Official Journal of the European Union, L 338, 1–26. **https://eur-lex.europa.eu/eli/reg/2005/2073/oj/eng**

3. Food Allergy Research and Resource Program. (2025). Food allergens – International regulatory chart. University of Nebraska-Lincoln. **https://farrp.unl.edu/IRChart**.

4. United States Department of Agriculture (USDA), Agricultural Marketing Service (AMS), Federal Grain Inspection Service (FGIS). **Mycotoxin Handbook**. Program Handbook. Washington, DC: USDA AMS; 2023. Available at: **https://www.usda.gov/guidance-documents/mycotoxin-handbook/ams/amsams-gd-2020-11-fgis-handbooks**

5. ISO 6887-1:2017. Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 1: General rules for the preparation of the initial suspension and decimal dilutions. **https://www.iso.org/standard/63335.html**

6. U.S. Food and Drug Administration. (n.d.). Hazard analysis and risk-based preventive controls for human food: Draft guidance for industry (Appendix 1: Potential hazards for foods). U.S. Department of Health and Human Services, Food and Drug Administration. **https://www.fda.gov/regulatory-information/search-fda-guidance-documents/draft-guidance-industry-hazard-analysis-and-risk-based-preventive-controls-human-food**

7. Marcel H. Zwietering, Liesbeth Jacxsens, Jeanne-Marie Membré, Maarten Nauta, Mats Peterz. Relevance of microbial finished product testing in food safety management. Food Control, 60, 2016, 31-43. **https://doi.org/10.1016/j.foodcont.2015.07.002**.

8. Stier, R. F. (2026, January/February). A practical guide to EMPs. Food Technology Magazine, 80(1). **https://www.ift.org/publications/food-technology-magazine/20262/january/columns/safety-and-quality-a-practical-guide-to-emps/**

9. International Commission on Microbiological Specifications for Foods. Usefulness of testing for Clostridium botulinum in powdered infant formula and dairy-based ingredients for infant formula. 2014. **https://www.icmsf.org/wp-content/uploads/2018/02/ICMSF_Infant_Formula_Testing_Revision1-20140117.pdf**

10. Canadian Food Inspection Agency. (2018). Safe Food for Canadians Regulations (SOR/2018-108). Government of Canada. **https://laws-lois.justice.gc.ca/eng/regulations/SOR-2018-108/**

11. Canadian Food Inspection Agency. (n.d.). Preventive control plan (PCP) interactive tool. Government of Canada. **https://inspection.canada.ca**

12. Codex Alimentarius Commission. (2020). General principles of food hygiene CXC 1-1969 (Revised 2020), Annex: Hazard analysis and critical control point (HACCP) system and guidelines for its application. FAO/WHO. **https://www.fao.org/fao-who-codexalimentarius/**

13. Food and Agriculture Organization of the United Nations, & World Health Organization. (2006). Food safety risk analysis: A guide for national food safety authorities (FAO Food and Nutrition Paper 87). **FAO. https://www.fao.org**

14. Global Food Safety Initiative. (2023). GFSI benchmarking requirements. The Consumer Goods Forum. **https://mygfsi.com**

15. Health Canada. (2019). *Policy on Listeria monocytogenes in ready-to-eat foods.* Government of Canada. **https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/policy-listeria-monocytogenes-ready-eat-foods.html**

16. International Commission on Microbiological Specifications for Foods. (2011). *Microorganisms in foods 8: Use of data for assessing process control and product acceptance.* Springer. **https://doi.org/10.1007/978-1-4419-9374-8**

17. International Organization for Standardization. (2018). *ISO 22000:2018 – Food safety management systems — Requirements for any organization in the food chain.* ISO. **https://www.iso.org/standard/65464.html**

18. U.S. Food and Drug Administration. (2015). *Current good manufacturing practice, hazard analysis, and risk-based preventive controls for human food (21 CFR Part 117).* U.S. Department of Health and Human Services. **https://www.ecfr.gov/current/title-21/part-117**

19. U.S. Food and Drug Administration. (2016). Hazard analysis and risk-based preventive controls for human food: Guidance for industry. U.S. Department of Health and Human Services. **https://www.fda.gov/food**

20. Salfinger, Y., Tortorello, M.L., American Public Health Association. 2015. Compendium of Methods for the Microbiological Examination of Foods (5th ed.). Washington, DC: American Public Health Association.

21. Almond Board of California. Preventing *Salmonella* Recontamination: Pathogen Environmental Monitoring Program Guidance Document. **https://www.almonds.com/almond-industry/processors-and-suppliers/processing-safe-product/pem**

Acknowledgement

Thank you to Fernando Sampedro, Ph.D., Food Safety and Risk Analysis Researcher at the University of Minnesota and international consultant for his contribution to this work and his continuous support.

Annex 1: Codex Alimentarius: maximum levels for mycotoxins in food

Mycotoxin	Food commodity	Maximum level (µg/kg)
Aflatoxins (total), includes AFB_1, AFB_2, AFG_1, AFG_2	Ready-to-eat peanuts	15
	Tree nuts (e.g., almonds, pistachios)	10
	Dried figs	10
	Dried chili pepper and nutmeg	20
Aflatoxin B_1	Ready-to-eat peanuts	10
	Tree nuts (e.g., almonds, pistachios)	8
	Dried figs	8
Aflatoxin M_1	Milk	0.5
Ochratoxin A	Cereals (wheat, barley, rye) and derived products	5
	Dried chili pepper, paprika, and nutmeg	20
Fumonisins B_1, B_2	Maize and maize-based products	4000
Deoxynivalenol (DON)	Wheat, maize, and barley	2000
Zearalenone	Maize and maize-based products	100
T-2 and HT-2 toxins	Cereals and cereal-based products	100
Patulin	Apple juice and ingredients in other beverages	50

Annex 2: User guide to use the FAO Mycotoxin sampling plan

https://tools.fstools.org/Samplingmodel/

Step 1: Open the tool

Go to the FAO Mycotoxin Sampling Tool page. The main tabs are:

- Instructions
- Edit Plans
- Chart Results
- Table Results
- Plan Summary
- Export To Excel

Step 2: Start in the instructions tab

Use this tab first to understand what the tool does. FAO explains the tool helps you evaluate sampling plan performance and choose a plan that minimizes lot misclassification risk while considering available resources.

Step 3: Go to Edit Plans

This is where you build the sampling plan you want to test. FAO states you can compare up to 10 different sampling plans at the same time.

In **Edit Plans**, fill in these fields:

A. Select the mycotoxin/commodity combination from the dropdown list, such as:
   - Aflatoxin/shelled corn (maize).
   - Or whichever aflatoxin/commodity option matches your case.

B. Enter the common parameters: These are the settings that apply to all plans you compare for that commodity. FAO lists three common parameters (kernel count per kg, regulatory limit, and analytical variance type).
   - **Kernel count per kg:** This is the number of kernels or units in 1 kg of product. The tool shows a default value for the selected commodity, but you can change it if your commodity differs from the default. For maize, if you do not have a better estimate, you can leave the default suggested by the tool.
   - **Regulatory limit**: Enter the legal or commercial maximum level that separates acceptable and unacceptable lots. FAO notes this can come from regulatory agencies, Codex, or industry requirements. (For your aflatoxin example, you might enter: 20 ng/g (ppb) if that is the limit you want to evaluate.)
   - **Analytical variance type:**
     - Within lab if one lab is used consistently.
     - Among lab if multiple labs are involved in the program.

FAO says the tool assumes among-lab analytical variance is double the within-lab analytical variance.

Step 4: Enter the plan-specific parameters

These are the parameters that define each sampling plan and let you compare alternatives. FAO identifies these plan-specific fields as: laboratory sample size, number of laboratory samples, test portion size, number of aliquots, and accept/reject limit.

A. Laboratory sample size (ns, kg): This is the smallest sample taken from the lot that will be ground in the mill for sample preparation. The tool converts this kg value into number of particles using the kernel count per kg.

Example:

- Plan 1 = 5 kg
- Plan 2 = 10 kg
- Plan 3 = 20 kg

This is a good way to compare how larger sample size reduces variability.

B. Number of laboratory samples (scnt): This is how many separate laboratory samples you will test. FAO notes this is important for attribute-type sampling plans, where all sample test results must be below the accept/reject limit for the lot to be accepted. There is no averaging of separate sample test results in that case.

Example:

- 1 laboratory sample
- Or 2 (if you want a stricter plan)

C. Test portion size (nss): This is the amount taken from the comminuted laboratory sample for extraction and analysis. The user guide shows examples comparing different test portion sizes because this can materially change the OC curve and reduce misclassification risk.

Example:

- 25 g
- 50 g
- 100 g

D. Number of aliquots (na): This is the number of aliquots quantified analytically. If more than one aliquot is entered, the tool assumes the aliquot measurements are averaged in the quantification process.

Example:

- 1 aliquot for a simple plan
- 2 aliquots if you want to examine whether more analytical replication helps

E. Accept/reject limit (Ca): This is the threshold used for the accept/reject decision. FAO states it may be equal to the regulatory limit, but buyers may also set it lower than the regulatory limit to reduce buyer's risk.

Example:

- Regulatory limit = 20 ng/g
- Accept/reject limit = 20 ng/g
- Or accept/reject limit = 18 ng/g (if you want a more conservative plan)

Step 5: Add more plans if you want to compare options

Click **Add a Plan** to create another row of plan-specific inputs. FAO says you can compare up to 10 plans this way.

This is the best part of the tool. Instead of guessing, you can directly compare things like:

- 5 kg vs 10 kg vs 20 kg laboratory sample size.
- 25 g vs 100 g test portion.
- 1 aliquot vs. 2 aliquots.
- Accept/reject limit equal to the legal limit vs. lower than the legal limit.

Step 6: Click Save Plans

Once the plan inputs are complete, click **Save Plans.** FAO says this is the step that locks in the sampling plan descriptions so the tool can evaluate them.

Step 7: Go to chart results

This tab shows the main outputs in graph form. FAO indicates this tab includes:

- Variance information.
- Variance ratios.
- OC curves.

What to look at here

A. Variance bars

The tool breaks total variability into:

- Sampling variance.
- Sample preparation variance.
- Analytical variance.

FAO explains that the step with the largest variance ratio is the one you should target if you want to improve the plan most effectively.

Practical reading:

- If sampling variance dominates, increase sample size or number of samples.
- If sample preparation variance dominates, increase test portion size.
- If analytical variance dominates, consider more aliquots or tighter analytical control. This interpretation follows directly from FAO's discussion of how changing ns, nss, na, and Ca affects plan performance.

B. OC curve

The OC curve shows the probability of accepting a lot at different true lot concentrations. FAO defines this as the probability that the sample test result is less than or equal to the accept/reject limit for a given lot concentration.

How to read it:

- At concentrations below the regulatory limit, rejection corresponds to seller's risk.
- At concentrations above the regulatory limit, acceptance corresponds to buyer's risk.

The steeper the curve around the limit, the better the plan is at separating good lots from bad lots. That is the practical takeaway from FAO's OC-curve framework.

Step 8: Adjust the chart controls if needed

In the chart area, you can control how much of the OC curve is shown. FAO notes that you can set things like the maximum lot concentration to compute and the minimum acceptance probability to plot, then click **Refresh** to redraw the graph.

Use this when the x-axis is too short or too long for your purpose.

Step 9: Open Table Results

This tab gives you the same information in numeric form instead of just graphs. FAO says it includes:

- Variance values.
- Variance ratios.
- OC-curve acceptance probabilities in table form (**tools.fstools.org**).

Set the table controls

You can define:

- Maximum lot concentration to compute.
- Lot concentration Increment.

Example:

- Maximum lot concentration = 40 ng/g
- Lot concentration increment = 5 ng/g

Then click Refresh to generate the table.

How to use this table

This is where you can read exact acceptance probabilities instead of estimating them visually from the OC curve. FAO notes the table gives acceptance probabilities, and reject probabilities can be obtained as 100% minus acceptance probability.

Example:

- If acceptance probability at 25 ppb is 35%, then rejection probability is 65%
- That means buyer's risk at 25 ppb is 35%

Step 10: Check Plan Summary

This tab gives a quick summary of the plan parameters you entered in **Edit Plans**. It is mainly a quality-control step so you can verify you didn't accidentally compare the wrong plan settings.

Step 11: Use Export To Excel

If you want to keep the results, graph them, or use them in a report or training slide, go to **Export To Excel**. FAO states this tab can export information from the **Chart Results** and **Table Results** tabs into an Excel spreadsheet.

Step 12: Interpret and improve the plan

FAO explains that buyer's and seller's risk can be changed by adjusting the sampling-plan design parameters: laboratory sample size, number of laboratory samples, test portion size, number of aliquots, and accept/reject limit. Increasing laboratory sample size, number of samples, test portion size, or number of aliquots reduces variability; lowering the accept/reject limit reduces buyer's risk but increases seller's risk.

Practical rule of thumb

If your plan is too weak:

- Increase laboratory sample size.
- Increase number of laboratory samples.
- Increase test portion size.
- Increase number of aliquots.
- Consider lowering the *accept/reject limit* only if you deliberately want a more conservative plan and accept the tradeoff of more false rejections.

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FS02715_0726