Control of *Salmonella* in Low-moisture Foods III: Process Validation and Environmental Monitoring

Part three of a three-part series

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ABSTRACT

Although low-moisture food products do not support *Salmonella* growth, the presence of low numbers of *Salmonella* can still cause illness. Therefore, the presence of the organism in low-moisture ready-to-eat foods must be prevented. To address the need for industry-wide guidance, the Grocery Manufacturers Association formed a *Salmonella* Control Task Force to develop guidance on the control of *Salmonella* when manufacturing low-moisture foods. Five of the control elements were covered in previous papers: preventing ingress or spread in a facility, controlling raw materials and ingredients, adhering to stringent hygiene practices in the Primary *Salmonella* Control Area, following hygienic design principles, and preventing growth in the facility by control of moisture. Here we address validation of control measures to inactivate *Salmonella* and verification of control through environmental monitoring.

SALMONELLA CONTROL ELEMENT 6: VALIDATE CONTROL MEASURES TO INACTIVATE *SALMONELLA*

When a lethality step is needed to inactivate *Salmonella* in a low-moisture product or ingredient, the processing parameters used should be adequate to inactivate the level of the organism likely to be present. According to the National Advisory Committee on Microbiological Criteria for Foods (NACMCF), validation encompasses collecting and evaluating scientific data and technical information to demonstrate that the control measures and associated critical limits at the lethality step, when followed, will result in a safe product (*43*). In addition, it is necessary to demonstrate that the chosen control measure and critical limits can be applied in production at a critical control point. Validation of lethality steps for low-moisture foods involves determining an appropriate log reduction for *Salmonella*, determining the critical limits in the process required to achieve the reduction, and confirming that the process equipment consistently delivers the critical limit parameters in the operation *(43, 52)*.

In general, NACMCF's definition for pasteurization *(44)* can be used to guide the determination of an appropriate level of log reduction. With respect to a low-moisture product, NACMCF's definition translates into applying any process, treatment, or combination thereof to reduce the most resistant *Salmonella* serotype "to a level that is not likely to present a public health risk under normal conditions of distribution and storage." NACMCF also indicated that a control measure aimed at inactivating the target pathogen does not protect the consumer if the product is subsequently recontaminated during manufacturing. The effective approach to prevent recontamination is through good hygiene practices verified by environmental monitoring (see Element 7) to ensure that recontamination is not likely to occur.

The level of reduction required will depend on the potential levels of *Salmonella*, if present, in the raw ingredients. Efforts have been made to set an appropriate level of log reduction for a specific low-moisture product based on a risk assessment. For example, a risk assessment *(16)* conducted to assess the risk of salmonellosis from almond consumption was used to determine that a 4-log reduction of *Salmonella* in raw almonds is adequate to ensure safety of the finished product *(6)*. In some instances, historical knowledge is used as the basis for validation *(49)*. For example, pasteurization at 72°C for 15 s is considered adequate to inactivate expected levels of vegetative pathogens of concern in raw milk. These parameters may be used as the critical limits or as the basis to establish other process parameters as critical limits at the lethality step to inactivate *Salmonella* in the fluid milk ingredient for a dried milk product; preventing recontamination after pasteurization during drying and subsequent handling would be essential to protect the finished dried product from recontamination. Both industry guidelines *(22)* and FSIS regulations in 9 CFR 590.575 *(12)* set parameters for the pasteurization

TABLE 1. Heat resistance of *Salmonella* in food matrices as influenced by a_w

Study	*Salmonella* serotype	Heating medium	Water activity (a_w)	Temperature (°C)	D-value (min)	z-value (°C)
Barrile and Cone, 1970 *(8)*	Anatum	Milk chocolate	Not reported	90	11	24.2
Harris, 2008 *(28)*	Enteritidis PT 30	Almonds (oil-roasted)	Not reported	121	0.85	27
		Almonds (blanched)	Not reported	70	1.0	29
Goepfert and Biggie, 1968 *(26)*	Typhimurium	Milk chocolate	Not reported[a]	70	816	19.0
				80	222	
				90	75	
	Senftenberg 775W	Milk chocolate	Not reported[a]	70	440	18.0
				80	116	
				90	36	
Archer et al., 1998 *(7)*	Weltevreden	Wheat flour	0.50-0.60[b]	69-71	80	30.3
				72-74	45	
				75-77	40-45	
			0.46-0.50[b]	69-71	55	53.9
				72-74	55	
				75-77	40-45	
			0.41-0.45[b]	69-71	55	19.6
			0.36-0.40[b]	72-74	75	15.2
				75-77	80	
			0.31-0.35[b]	69-71	345	29.2
				75-77	85	
			0.25-0.30[b]	69-71	165	34.7
				72-74	240	
				75-77	150	

TABLE 1. *Continued*

VanCauwenberge et al., 1981 *(56)*	Newington	Corn flour (15% moisture)	Not reported	49	18	Not reported
	Typhimurium				48	
	Kentucky				66	
	Anatum				48	
	Senftenberg				300	
	Cubana				150	
	Thompson				264	
	Tennessee				594	
	Senftenberg	Corn flour (10% moisture)	Not reported	49	366	Not reported
	Anatum				156	
Liu et al., 1969 *(36)*	Senftenberg 775W	Animal feed[c] (15 % moisture)	Not reported	71.1	10.0	10.4
		Animal feed[c] (10 % moisture)	Not reported	71.1	115.2	11.0
Sumner et al., 1991 *(53)*	Typhimurium	Chocolate syrup	0.75	65.6	2.7	8.3
			0.83 (product A)	65.6	1.2	6.2
			0.83 (product B)	65.6	3.2	7.7
			0.84	65.6	2.7	8.3

[a] Moisture level probably less than 2.5%.

[b] Value of a_w measured after drying the inoculated wheat flour.

[c] Simulated-naturally contaminated meat and bone meal stabilized at the indicated moisture level was used in the study.

of dried egg white, which include heating the product in a closed container to at least 130°F (54.4°C) for 7 days or longer until *Salmonella* is no longer detected (as a practical matter, the egg industry routinely uses a more severe heat treatment in order to eliminate the avian influenza virus as well as *Salmonella*).

Both thermal and non-thermal control measures can be used for *Salmonella* inactivation to achieve the target log reduction. Various processing steps (e.g., cooking, frying, roasting, baking, heat extruding, fumigation) may be used to inactivate *Salmonella* in a low-moisture product. Thermal processing is the most commonly used control measure to inactivate *Salmonella*. For example, the Almond Board of California's Technical Expert Review Panel (ABC TERP) determined that oil roasting at or above 260°F (126.7°C) for 2 min will result in a 5-log reduction of *Salmonella* on the surface of whole almonds *(1)*. The ABC TERP also provided minimum time and temperature combinations required for blanching processes to deliver a 4 or 5-log reduction of *Salmonella* on almonds *(1)*. These parameters were determined on the basis of heat resistance data for *Salmonella* Enteritidis PT 30 as the target organism.

It is useful to review available scientific data for the processing method of interest, including high temperature short time or low temperature long time, when desirable for maintaining product quality. In order to assure appropriate validation, it is also necessary to evaluate scientific and processing equipment data and information specific to the processing technology under consideration. A process authority should be consulted where necessary. For example, the ABC TERP, which consists of experienced microbiologists and processing experts, evaluates the adequacy of various treatments to inactivate *Salmonella* in raw almonds and develops guidelines for validating individual processes, including propylene oxide (PPO) treatment for raw almond kernels, PPO treatment for in-shell almonds, blanching, oil roasting, dry roasting and other processes that may be proprietary *(1)*.

Heat resistance of *Salmonella* is affected by factors during heating, as well as the *Salmonella* strains used *(28)*. Heat resistance observed in an aqueous system may not be applicable to a low-moisture product. For example, a study by Ng and colleagues *(46)* found that *S.* Senftenberg 775W was the most heat resistant among 300 strains evaluated in an aqueous solution, while this strain was found to be less heat resistant than *S.* Typhimurium in chocolate *(26)*. *S.* Enteritidis PT 30, the target organism for raw almonds, was implicated in a foodborne illness outbreak and was found to be more resistant to dry heat than many of the strains evaluated on almonds *(1, 58)*.

A number of studies have been published on heat resistance of *Salmonella* in various low-moisture products. Available D- and z-values for heat resistance of various *Salmonella* strains in low-moisture matrices are shown in Table 1 for food matrices and in Table 2 for model systems. These data indicate that heat resistance is much greater in a product with low a_w than in a high-moisture product. For example, while reaching an internal product temperature of 160°F (71.1°C) without a hold time would eliminate *Salmonella* in raw poultry *(23)*, the same temperature would result in little inactivation of *Salmonella* in milk chocolate, in which the D-value for *S.* Typhimurium has been reported as 816 min at 71°C *(26)*.

Table 1 shows D-values for *Salmonella* in wheat flour *(7)*, milk chocolate *(8, 26)*, almonds *(28)*, corn flour *(56)*, and dry animal feeds *(36)*. In addition, recent research *(18)* found that, based on the non-linear Weibull model, 42 ± 8 min at 90°C achieved a 5-log reduction of a mixture of three outbreak-associated *S.* Tennessee strains in peanut butter (49 ± 12 min were needed to inactivate a composite of other *Salmonella* isolates). Liu et al. *(36),* who determined the heat resistance of *S.* Senftenberg 775W in meat and bone meal and chicken starter at moisture levels from 5% to 30%, found that the method used to prepare the inoculum (growing the cells in a laboratory medium vs. in meat and bone meal suspension) affected the heat resistance. Akinleye *(3)* reported that D- and z-values were affected by water activity of a salt solution model system. D- and z-values relevant to low-moisture heat conditions from this study are shown in Table 2, along with data from another study using sucrose as a model system *(53)*. It should be noted that comparison of inactivation kinetics data from different studies can be difficult, and it is crucial to review the raw data and experimental procedures, as well as the D- and z-values reported, so as to apply the data appropriately.

Heat-inactivation of *Salmonella* in low water activity matrices was found be non-linear in many cases, such as in peanut butter *(37)*, oil-roasted almonds *(2)*, flour *(7)*, and laboratory media *(39)*. The *Salmonella* inactivation curve in low water activity foods can be complex, often showing a concave upwards curvature, and significant tailing has been observed *(28, 38, 39)*. Thus, the rate of inactivation may not be constant throughout the heating process, and caution must be used when interpreting and using heat resistance data to support the adequacy of the process parameters.

In a study by Archer et al. *(7)* of the heat resistance of *Salmonella* Weltevreden in wheat flour, the investigators observed that death kinetics were non-linear, with approximately a 1-log reduction in the first 5–10 minutes of heating, followed by a slower, linear decrease in survivors. To be conservative, the investigators calculated the D-value based on the second, slower phase of the inactivation curve. Sumner et al. *(53)* reported that the D-value of *Salmonella* Typhimurium ATCC 13311 increased by more than 100-fold as the a_w was reduced from 0.98 to 0.83 in sucrose solutions; this trend was observed in the treatment temperature range of 65 to 77°C (149–170.6°F); the study did not investigate temperatures below 65°C for *Salmonella* inactivation. In laboratory media with a_w adjusted with glucose and fructose, Mattick et al. *(39)* reported that *Salmonella* Typhimurium DT104 inactivation was non-linear in the range of 55 to 80°C (131–176°F). At temperatures ≥ 70°C (158°F), heat resistance increased as the a_w decreased from 0.90 to 0.65; however, this trend was not observed for heat treatment at 65°C (149°F) or below, at which range decreasing a_w from 0.90 to 0.65 either had little effect or slightly decreased the heat resistance of *Salmonella*.

Some studies have also been published on the inactivation of *Salmonella* by non-thermal processing. For example, the efficacy of low-energy X-ray irradiation was examined for inactivating *S.* Enteritidis PT 30 on almonds at different water activities *(34)*. The organism was found to be more resistant at a_w 0.65 (D_{10}-value ~ 0.34 kGy) compared to a_w 0.23 (D_{10}-value ~0.26 kGy). Irradiation, for products where its use has been approved, can also be an effective control measure. Irradiation with a dose up to 30 kGy (21 CFR 179.26) has been approved for use in inactivating microorganisms in dry aromatic vegetable substances such as herbs, spices and vegetable seasonings

TABLE 2. Heat resistance of *Salmonella* in model systsems as influenced by a_w

Study	*Salmonella* serotype	Heating menstruum	Water activity (a_w)	Temperature (°C)	D-value (min)	z-value (°C)
Akinleye, 1994 *(3)*	Typhimurium	Salt solution	0.42	90	32.3	30.3
				100	12.5	
				110	18.2	
				120	8.9	
			0.31	90	20	40
				100	12.7	
				110	16.7	
				120	10.6	
Sumner et al., 1991 *(53)*	Typhimurium	Sucrose solution	0.83	65.5	40.2	7.6
			0.85	65.5	19.2	6.5
			0.89	65.5	4.8	6.9
			0.94	65.5	1.4	7.7
			0.98	65.5	0.29	7.6

(13). Danyluk et al. *(17)* reported a greater than 5-log reduction of *S.* Enteritidis PT 30 on almonds after the product had been treated with PPO (0.5 kg/m^3) for 4 hours, followed by storage for 5 days. Ethylene oxide is effective for treating spices and herbs to eliminate *Salmonella (47, 57)*. While its application as a control measure is being phased out in some cases (such as for basil), it remains an effective measure to eliminate *Salmonella* in spices and herbs where approved, especially for treating high-risk ingredients that otherwise would not receive a lethality treatment for *Salmonella*.

Validation testing can be carried out using *Salmonella* (appropriate strains), or a surrogate organism that has been validated for the product and process under consideration, or a non-microbial method such as using an enzyme as a surrogate that has been validated for use in such applications. When the time and temperature profiles of a process can be mimicked in the laboratory (e.g., oil roasting), a challenge study with appropriate *Salmonella* strains can be conducted in the laboratory to validate the process *(35)*. This approach has been used to validate a dry-air roasting process for peanuts, where a lab-scale roaster was used to mimic the actual processing times and temperatures, and the process was found adequate to deliver a 4-log reduction of several *Salmonella* strains *(55)*.

When it is difficult to mimic the processing conditions in the laboratory with sufficient accuracy, a surrogate organism or a non-microbial substance may be used for validation. When a surrogate organism or substance is used, a relationship between the target *Salmonella* strain and the surrogate needs to be established, and the surrogate should behave in a way that a correlation can be made in a conservative manner *(35)*. In practice, a surrogate that has heat resistance comparable to or greater than the target *Salmonella* strain (to build in a margin of safety) is usually selected. For example, studies in several laboratories were conducted to select a surrogate organism for *S.* Enteritidis PT 30, the pertinent pathogen for almonds *(58)*. Correlation between *S.* Enteritidis PT 30 and a surrogate organism, *Enterococcus faecium* NRRL B-2354 (also known as *Pediococcus* spp. NRRL B-2354), has been established for dry heat in the 250 – 310°F (121.1 – 154.4°C) range for almonds. *E. faecium* NRRL B-2354 was found to have inactivation characteristics comparable to *S.* Enteritidis PT 30 under dry heat conditions *(11, 58)*. In fact, the D-values for the surrogate were slightly higher than those for the pathogen in the 250 – 310°F (121.1 – 154.4°C) range for almonds subjected to dry heating.

Alternatively, particles containing enzymes can be passed through a plant processing step and tested for residual enzyme activity, thus providing an indication of process lethality. The use of enzymes for process validation has been described for various thermal processes *(10, 54)*. Testing for phosphatase has been used to verify that the pasteurization of milk has occurred.

Common industry practices

- Determine the target level of *Salmonella* reduction in the product and process under consideration.
  - The determination can be based on the rationale outlined by NACMCF *(44)*. The target level of *Salmonella* reduction should be such that the treated product presents a reasonable certainty of no harm to the consumer.
  - A targeted 2- to 5-log reduction is commonly selected on

the basis of a hazard analysis that includes historical association of ingredients with *Salmonella*, prevalence and extent of contamination (i.e., the incoming load of *Salmonella*), and the intended use of the final product. The selected log reduction should include a margin of safety, e.g., an additional 2-log reduction beyond the extent or levels of contamination expected to occur in the ingredients *(21, 25, 41, 42)*.

- Where regulatory or industry standards for log reduction have been established, these should be applied. For example, based on a comprehensive risk assessment a 4-log reduction of *Salmonella* in raw almonds has been established in the US to ensure safety of the finished product.

❑ Determine the adequacy of the selected control measure and associated critical limits for processing.

- Critical limits should be developed on the basis of thermal parameters (e.g., D- and z-values, thermal death times) or non-thermal parameters of the most resistant and pertinent *Salmonella* serotype, based on occurrence in the product ingredients, processing environment, and/or association with an outbreak involving the product or similar products.
- In many cases, processing conditions are initially driven by quality attributes, and it is essential to determine whether these conditions can deliver the target log reduction (several quick trials in the lab can be done for a feasibility assessment; literature data can also be used). Working with process engineers to optimize the process to deliver the target log reduction while still maintaining product quality is a common approach used in the industry.
- In practice, several approaches can be used for validating the adequacy of process parameters. As noted previously, if the process can be mimicked reasonably well in a laboratory (e.g., for oil roasting), then *Salmonella* can be used in process validation in a laboratory setting to confirm that the critical limits, when achieved, consistently result in the target *Salmonella* log reduction. If the process is too complex to mimic in a lab setting (e.g., heat extrusion), other approaches for validation may be used, such as determining lethality based on the processing conditions (e.g., integrated lethality based on time and temperature profiles) or using a suitable surrogate for validation on the processing line. In addition to process parameters, other critical factors such as the initial temperature and initial moisture level of the ingredient(s) should also be considered in lethality validation studies.
- A non-pathogenic microbial surrogate or a non-microbial surrogate such as an enzyme can be used after appropriate validation. For example, *E. faecium* NRRL B-2354 has been determined to be an appropriate surrogate for *Salmonella* in the validation of processing methods for almonds *(1)*.

❑ Use published data to guide the determination of whether a challenge study is needed for control measure validation.

- The utility of literature data depends on the food or model matrix and the design used in the study to generate the data. According to the rationale outlined by NACMCF *(44)*, the value of a particular set of literature data will be enhanced if the matrix and conditions used to generate the data are similar to the product and process to which the data are being applied.
- Available heat resistance data may be used to estimate log reduction by thermal processing in a low-moisture product. The ideal approach is to use available heat resistance data collected in the same food matrix, such as using D- and z-values obtained in wheat flour to calculate log reduction in wheat flour during heat processing. Care should be taken when using D- and z-values, as inactivation may not be linear. In some cases a non-linear heat resistance model may have been developed for a product (e.g., peanut butter, almonds) and this can also be used. When D- and z-values are not available for the food at the water activity under consideration, data obtained with a product of similar composition may be used, e.g., data obtained in wheat flour or corn flour for cereal products. When data for a food matrix are not available, data obtained in a model system (e.g., sucrose solution) with similar a_w may be used to estimate lethality. When using this approach, it is important to keep in mind uncertainties inherent in applying available data and assumptions made.
- In most cases, literature data are used to guide efforts in identifying parameters specific to a product of interest, whether a challenge study is needed, and how a challenge study may be designed. Whether published data are sufficient to support the adequacy of the lethality of a chosen control measure and associated critical limits depends on several factors. According to the rationale developed from industry experience *(49)*, if an evaluation based on literature data shows that survival of *Salmonella* is not likely to occur, with a reasonable margin of safety, challenge studies would not be needed. For example, analysis of the time and temperature profiles for a heat extrusion process may indicate that, based on the a_w of the ingredients and the product, the process is expected to deliver *Salmonella* inactivation that would greatly exceed 5 log. On the other hand, if there is less confidence in using published data, then limited challenge studies may be needed to verify estimated log reduction based on literature data. If the evaluation shows that there is limited lethality for the product/process based

on available heat resistance data, then additional studies or process re-design would be warranted.
  - Available scientific guidance, such as the NACMCF guidance on parameters for performing an inoculated pack/challenge study *(45)*, should be used for validation of control measures through microbiological challenge testing.
  - Microbiological expertise is necessary to determine the relevance and validity of applying published data to a specific product and process. An experienced microbiologist or process authority should assist in the use and interpretation of published data.
- ❑ Consider both thermal and non-thermal control measures, with validation, to eliminate *Salmonella*.
  - Thermal processing can be used under dry or moist conditions. Moist heat treatment is followed by a drying step in the manufacturing of many low-moisture products. Where appropriate (e.g., for some spices and seeds) a combination of steam treatment (pressurized or non-pressurized) and drying may be used to inactivate *Salmonella*. In such cases, validation should focus on determining the lethality of the steam process alone as a conservative scenario or, if heating after the steam process is included in lethality calculations, the combined effects of the multiple processing steps should be validated.
  - Validation should focus on the CCP used to deliver the target log reduction, when one of multiple steps effecting lethality is chosen as the CCP. Cumulative effect from multiple inactivation steps may be used to achieve the target log reduction, even though individual steps alone are not sufficient to achieve the target lethality, as long as the individual processing steps and the combined lethality are validated. Be aware that not all heating steps in a process will provide *Salmonella* inactivation. For example, spray drying is an evaporative cooling process that usually does not result in appreciable inactivation. Another example of minimal to no *Salmonella* inactivation may be a finishing dryer following the heat extrusion process.
  - For a low-moisture product (e.g., spray-dried milk) that starts with high-moisture ingredients (e.g., milk), the heat treatment process prior to drying should be readily verifiable, and efforts should be concentrated on preventing post-lethality contamination during drying and the subsequent steps through finished product packaging.
  - Examples of non-thermal control measures are treatment with an approved chemical for fumigation, such as propylene oxide or ethylene oxide, and treatment with irradiation.
- ❑ Once the lethality of the process is validated by scientific data, it should be ensured that the operation can deliver the critical limits and that the parameters are consistently met, through in-plant validation, which is an integral part of the validation process. Subsequently, verification of process control may include activities such as records review, calibration of instruments, and periodic finished product testing or other type of independent checks.
- ❑ It should also be ensured that raw material/ingredient suppliers validate their processes and the control measures.

SALMONELLA CONTROL ELEMENT 7: ESTABLISH PROCEDURES FOR VERIFICATION OF *SALMONELLA* CONTROLS AND CORRECTIVE ACTIONS

The adequacy of the *Salmonella* control program should be verified on an ongoing basis to assure effectiveness and to drive continuous improvement. Verification should focus on implementing a robust environmental monitoring program that has been designed to identify transient and/or resident *Salmonella* in the processing areas. Appropriate corrective action procedures must be developed to address positive *Salmonella* findings with the intent of containing the contamination, identifying the potential source, and eliminating the problem. This section focuses on environmental monitoring and corrective actions to be taken when *Salmonella* is found in the environment, since this is one of the most important verification activities in low-moisture product manufacturing. Other verification activities, such as those for critical control points in a HACCP system, are well covered elsewhere *(9, 33, 43, 51)*.

Environmental monitoring is an essential component for *Salmonella* control, as it provides a microbiological assessment of a plant's environment and an assessment of the effectiveness of sanitation and the overall *Salmonella* control program *(27, 40, 59)*. Environmental monitoring is not, in itself, a control measure. Rather, it is a tool to verify the effectiveness of the overall *Salmonella* control program. Monitoring results provide critical information to improve *Salmonella* control in the plant environment; this information should be used to correct problem areas before they pose a risk to finished product. With this understanding, it is critical that the program be designed and implemented so as to maximize detection of *Salmonella.* A robust environmental monitoring program is one of many prerequisite programs that together provide a firm foundation for effective food safety management.

The target organism for environmental monitoring for low-moisture foods should be *Salmonella*. Scientific literature suggests that the pathogen is more persistent in the environment than other organisms such as coliforms and Enterobacteriaceae. A suitable indicator for *Salmonella* has not been identified *(19)*. Testing with enumeration of Enterobacteriaceae, however, may help assess moisture control in areas in the processing environment intended to remain dry *(30)*. Enterobacteriaceae is a useful indicator of process hygiene and may be monitored in parallel as a hygiene indicator for verification of general sanitation effectiveness. However, it cannot be a substitute for the direct monitoring of *Salmonella* because, while high levels of Enterobacteriaceae suggest an increased risk for the presence of *Salmonella*, low levels of Enterobacteriaceae do not guarantee absence of the pathogen *(15, 19)*.

Environmental monitoring for *Salmonella* is generally conducted on non-product contact surfaces (non-PCSs). Non-PCSs in the Primary *Salmonella* Control Area (PSCA) should be the main focus of routine monitoring for *Salmonella*. However, environmental monitoring for *Salmonella* should also be conducted in other areas of the facility (e.g., wet processing or handling of raw materials).

TABLE 3. Example of an environmental monitoring program for production of low-moisture foods

Sampling Zone	Definition	Examples of Sample Sites*	Test for	Frequency	Number of Samples**
Zone 1	Product contact surfaces (PCS) in the Primary *Salmonella* Control Area	Conveyors, filler hoppers, scrapers/utensils, packaging equipment, etc.	Indicator organisms (e.g. Aerobic Plate Count; Enterobacteriaceae); *Salmonella* only when special circumstances dictate	Post-Sanitation or as needed for investigational, validation, or verification purposes	Line Dependent
Zone 2	Non-PCS within close proximity to PCS in Zone 1. - Areas that, if contaminated, could reasonably lead to PCS contamination (i.e., under normal operational practices)	Exterior of equipment, legs/frameworks, motor housings, catwalks, control panels, scrap carts, floor drains, HVAC vents, vacuum cleaners if used near PCSs, air filters, weight scales, floor mats at packaging, etc.	*Salmonella*	Weekly, Biweekly, or Monthly	5-10
Zone 3	Non-PCS within process area but more removed from PCS. - Areas that, if contaminated, could not reasonably lead to PCS contamination without mechanical or human intervention (i.e., employee using compressed air to clean floors or a piece of equipment being moved)	Cleaning tools (brooms, squeegees), floor scrubbers, forklifts, floor drains, traffic pathways into process area, ceiling drain pipes, wall/floor junctures, wash stations, ingredient storage areas, etc.	*Salmonella*	Weekly or Monthly	3-6
Zone 4	Non-PCS outside processing areas. - Areas that, if contaminated, could spread to the processing area via foot or equipment traffic (i.e. waste carts picking up contamination in compactor room)	Compactor areas, employee entrances, locker rooms, storage rooms, labs, etc.	*Salmonella*	Monthly or Quarterly	2-4

* It is recommended that a facility assessment be done to identify sampling sites, in order to include potentially problematic areas. Weekly monitoring may be considered as a starting point to establish a solid baseline and the frequency may be revised based on results over time.

** In general, a greater number of samples are taken in Zone 2 than Zone 3 and in Zone 3 than Zone 4 – a ratio of 5:3:2, 6:3:1, 7:2:1, 8:1:1 have been used depending on the product and process, although other approaches may be effective. A larger facility with multiple process lines may take a greater number of samples than those indicated for the zones.

Monitoring in these areas can provide insight into the potential for *Salmonella* to be present and potentially spread into the PSCA. Within the PSCA, non-PCS areas adjacent to PCSs should be monitored with relatively high frequency. If these areas are not maintained in sanitary condition, they may pose a risk of product contamination. Non-PCSs within the PSCA that are more distant from PCSs should be sampled with medium to high frequency, and non-PCSs outside the PSCA should be sampled with low to medium frequency (Table 3). Each facility should determine the frequency adequate for its product and process. In general, high, medium and low frequency would correspond to daily/weekly, monthly, and quarterly testing, respectively.

Testing of a PCS and finished product may be done under some circumstances as part of the overall verification of *Salmonella* control. PCS testing may play an important role in hygienic qualification for equipment prior to use or for investigation of positive *Salmonella* findings. Periodic product testing can be useful in verifying that the food safety system for *Salmonella* control is working. Sampling plans used by the industry for product testing include those described in the FDA BAM *(4, 5)* and those described by ICMSF *(29)*. However, because it has well-known limitations in finding low levels of contamination, product testing alone is not a reliable means for assuring the absence of *Salmonella (29)*.

An adequate number of samples should be taken at appropriate frequencies for the environmental monitoring program to be effective. The number of samples and the frequency of sampling depend on the operation and facility. The sampling frequency can, in part, be based on current industry practices.

The first step in developing the frequency of testing and the test sites in an environmental monitoring program is to establish a solid baseline. Weekly monitoring may be considered as a starting point and the frequency revised based on the results over time. For example, in a facility that has historical testing data that show consistent *Salmonella* negatives in the environment based on a rigorous sampling program, the monitoring frequency can be reduced. On the other hand, a facility should be prepared to increase monitoring when changes in the operation warrant more monitoring, e.g., ingredient changes, leaky roof, drain back up, construction events, equipment installation, or detecion of *Salmonella* during routine environmental monitoring.

An official or validated method, such as the FDA BAM *Salmonella* method *(5)* or ISO 6579 *(32)*, should be used for testing. For some products, methodology may need to be modified and validated, as some food components (e.g., high fat levels) can complicate the sample preparation and pre-enrichment step and other aspects of the analysis. Both methods include a section on the testing of environmental samples. An alternative method may be used after it is validated as equivalent in sensitivity and specificity to a standard reference method for environmental samples or for the product being tested. Choosing a validated method is important, because a method validated for one purpose may not be suitable for another purpose; similarly, a method validated for individual sample units may not be suitable for testing sample composites *(40)*.

Common industry practices

- ❑ Develop a written program for routine environmental monitoring.
  - – The program should include elements such as identification of sampling sites, frequency of sampling, number of samples, sampling procedure, and test

method. Examples of these elements are described in Table 3. Corrective actions to be taken when a positive is found should also be outlined (see examples in Table 4).

– Sampling devices noted in the program should be appropriate for the types of samples collected and validated as necessary. For example, if sponges are used, they must not contain preservatives, and validation of *Salmonella* recovery is recommended.
– Sampling sites should be delineated into zones to facilitate program development, provide focus to critical sampling areas, and help direct appropriate corrective actions. For example, four zones may be established:
  • Zone 1 for PCSs in the Primary *Salmonella* Control Area;
  • Zone 2 for non-PCSs adjacent to or within close proximity to PCSs in the Primary *Salmonella* Control Area;
  • Zone 3 for non-PCSs more distant from PCSs in the Primary *Salmonella* Control Area and process areas outside the Primary *Salmonella* Control Area; and
  • Zone 4 for areas outside the process area (e.g., employee entrance, locker room, warehouse, loading dock).
– Routine environmental monitoring should target testing non-PCSs under normal operating conditions. Samples taken post-sanitation provide sanitation verification only and would not meet the true intent of environmental sampling. A "seek and destroy" philosophy should be adopted in environmental monitoring. This means that the monitoring program is designed to aggressively search for *Salmonella*, particularly in environmental sites where *Salmonella* might be expected to be present, might concentrate, or might grow and spread. Table 5 provides examples of potential *Salmonella*-positive sites, based on food industry experience; the listing is by no means inclusive of all potential sites.
– Using only preset sample sites is not recommended, since it significantly limits the scope of sampling and will likely miss emerging areas of concern. However, some sites may be sampled on a continuing basis to assess trends. Sampling data should be reviewed on a routine basis. The sampling program should be dynamic and responsive to the data generated.
– A rotation schedule should be developed to allow all areas of the plant to be sampled on a periodic basis, e.g., weekly monitoring with rotation of sites between different areas of the plant, with all sites sampled within a specified time period (e.g., monthly or quarterly). However, this should not be set up in a manner that excludes the sampling of an area of concern identified in a "non-scheduled" area. The sampling plan should be flexible and allow for additional samples to be collected where appropriate.

❑ Increase environmental monitoring (frequency and/or number of samples), as well as other control measures, in response to plant events such as during and after construction, and after equipment installation and major repairs are completed. An example of intensified control and monitoring is shown in Table 6.

❑ Develop a policy on whether and when to test PCSs and/or finished product and a program for this testing.
– Testing of PCS, if included in the program, should be done only after a policy has been established with regard to the impact of a PCS-positive on finished product and the actions to be taken. Routine testing of PCSs is not particularly meaningful in verification because, given an effective *Salmonella* control program, contamination, if any, is likely to be sporadic, and sampling is unlikely to find positives on PCS.
  • PCS testing may be done as part of corrective actions for an environmental positive, e.g., in sampling for investigational purposes following positive *Salmonella* findings in areas that may pose a risk for PCS contamination on the line (see Table 4). PCS testing may also be valuable under other circumstances, such as hygienic qualification of a piece of equipment prior to use in production, e.g., for new equipment or newly-acquired equipment that has been used in another facility.
– Manufacturers should decide whether or not to conduct finished product testing based on an evaluation of risk. Customer requirements (i.e., Certificates of Analysis) may also dictate the need for finished product testing.
  • Whenever finished product testing is performed, the tested lot should be isolated, placed on hold, and released into commerce only if the product tests negative for *Salmonella*.
  • **If a product sample tests positive for *Salmonella*, the tested lot is considered adulterated and should not be released into commerce. As noted previously, retesting should not be conducted for the purpose of negating the initial test results (*31, 48*). Resampling almost always increases the chance of accepting a contaminated lot. The lower the prevalence level of *Salmonella* in the product, the more difficult it will be to confirm, and it is virtually impossible to confirm very low prevalence by resampling *(31)*.**
  • Retesting for investigational purposes only (i.e., to try to determine the level or incidence of contamination in the sample) may be appropriate.
  • The lot associated with a positive sample may be reworked using a validated inactivation step. In addition to product disposition, other corrective actions may be taken as appropriate (see below).

TABLE 4. Examples of corrective action procedures following positive *Salmonella* findings in the plant environment

Zone 2, 3, or 4: Response to a Single Positive

Corrective actions must be taken when a *Salmonella* positive is found in any zone. Corrective actions should be initiated based on presumptive positive test results. The actions should aim to eliminate potential sources of the contamination.

Corrective actions common to Zone 2, 3, and 4 may include the following:

∞ Initiate pre-assigned response team to conduct a preliminary investigation to determine potential cause or source for the contamination (e.g., water leaks, maintenance activity, construction, etc.). The suspect site and surrounding areas should be examined as part of the investigation.

∞ Take immediate actions to correct any GMP deficiencies based on findings. These may include:

- Quarantine the suspect area and limit access to the area.
- Reinforce hygienic practices with appropriate employees (retrain if necessary).
- Re-examine cleaning frequencies and revise as appropriate.
- Eliminate water and water collection points, if present.
- Repair damaged floors/walls and other structural damage as appropriate.
- Re-examine traffic patterns. Where necessary and feasible, limit traffic flows (both employees and mobile equipment) through the area, restrict fork truck movement, redirect high risk traffic patterns from adjacent areas, etc.

∞ If desired, conduct investigational sampling of the suspect and surrounding areas prior to cleaning. Precaution should be taken to avoid spreading potential contamination from the suspect area to other areas in the plant.

∞ Thoroughly clean/sanitize and dry the positive site and the surrounding area. Use dry, controlled wet, and/or wet cleaning as appropriate, according to guidelines described in Element 4 *(14)*.

∞ Re-sample the implicated area and other sites within the surrounding and traffic pattern areas. If the positive is found in Zone 3, Zone 2 sites in the implicated area should be sampled and tested to verify that contamination has not spread to areas closer to PCSs; if the positive is in Zone 4, all Zone 3 sites close to the implicated area should be sampled and tested to verify that contamination has not spread into the process area.

∞ Increase sampling frequency, e.g., from weekly to once every two days in Zone 3, from weekly to daily for Zone 2. After 3 consecutive negatives, the routine sampling frequency and rotation plan for the *Salmonella* monitoring may be resumed.

Zone 4 areas are remote from production and generally present low risk to product. However, results from Zone 4 do provide information about the non-production environment and traffic flow. Although it is expected that *Salmonella* may be found occasionally in Zone 4, a positive finding should prompt additional actions beyond routine sanitation.

A Zone 3 positive, in the absence of a Zone 2 positive, is an early indicator of a sanitation program that is not robust enough. The implicated process may or may not be suspended based on the positive location and its proximity to product contact surfaces.

Zone 2: Additional Actions for a Single Positive

∞ Stopping production for sanitation may be appropriate under certain circumstances where finishe product or PCSs may be at risk.

∞ Whether or not to disassemble the line depends on the equipment associated with the positive site and how close the site is to finished product. Breaking down the line may not always be warranted if cleaning and re-sampling can be conducted without affecting PCSs. For example, the outside of a cooling tunnel and support frames may fall into a Zone 2 sampling category, and these sites should not affect product contact surfaces or cause the line to be broken down. However, if deemed necessary, break down the line from the positive site on, and disassemble equipment as necessary to ensure all PCSs are accessible for cleaning and sanitation. Thoroughly clean, sanitize, and dry the line and the surrounding areas starting from the positive site through the end of the line.

TABLE 4. *Continued*

- ∞ Conduct pre-operational inspections on the line equipment and in the area as applicable. Include Zones 2 & 3, and possibly Zone 1, as necessary in the sampling plan to re-qualify the line. Pre-operational test results should be obtained and confirmed negative prior to start-up if Zone 1 samples are included.
- ∞ Product testing may or may not be necessary depending on where the positive site was located. If finished product testing is already conducted as part of the overall food safety program (e.g., products with a *Salmonella* specification), intensified product testing may be initiated following any Zone 2 *Salmonella* positive finding. For example, the stringency of the sampling plan may increase from a plan with 3 samples of 25 g each to a case 11 (n = 10), case 14 (n = 30), or case 15 (n = 60), depending on the situation, with c = 0 in all cases; or from testing a 375 g composite to testing 2 × 375 g (750 g) or 4 × 375 g (1500 g). Whenever a product lot is subjected to testing, the lot should be held and released only if the test result is negative for *Salmonella*.

Special Circumstances: Consecutive Positives (all Zones)

When a sound control program for *Salmonella* is in place, finding multiple and/or consecutive positives may indicate that the primary source is a harborage site, where the organism may have become established and is multiplying. This can lead to an increased risk for spreading the organism and ultimately process line contamination. Corrective actions outlined below may be followed for problem resolution.

- ∞ Map the contamination sites on a layout of the facility to aid in locating the source of contamination, or at least suggest additional sites to sample. It is critical that a harborage site, if one exists, be found and eliminated. This usually means taking more samples than those taken during routine monitoring in the affected and traffic flow areas.
- ∞ Reinforce GMP training and hygienic practices and provide additional attention to sanitation procedures.
- ∞ Visually inspect areas for potential niches. Intensify cleaning activities around these areas.
- ∞ Visually inspect handling practices (production, sanitation, maintenance, material handling) and correct non-hygienic employee practices.
- ∞ Review equipment cleaning and preventative maintenance protocols and revise if necessary.
- ∞ Examine processing equipment and consider equipment redesign if necessary.
- ∞ PCS or product testing may be necessary or need to be intensified for Zone 2 consecutive positives. In some operations, testing may involve testing of worst-case samples on the line, e.g., sifter tailings on a spray dryer system. Line samples may be taken at various times and/or from various locations to help pinpoint potential contamination sites. Investigational samples should be analyzed individually, not as composites.
- ∞ Depending on the location of the positive, consideration should be given to testing Zone 1 sites. For example, consideration should be given to testing Zone 1 sites (i.e., PCSs) as a response to multiple positives in Zone 2. Consideration may also be given to Zone 1 testing under other circumstances, such as qualification for new equipment or relocated equipment, positive product tests or implication of products by epidemiologic investigations in an outbreak.

- ❑ An official or validated method should be used to test samples taken from the environment or finished product.
  - – The FDA BAM method *(5)* and the ISO 6579 method *(32)* apply to various products described in the methods, as well as to environmental samples. The FDA BAM method and the ISO 6579 method are considered the official method in the US and EU, respectively. A method that has been validated to be equivalent in specificity and sensitivity to one of these official methods may also be used. According to the FDA *(5)*, a validated rapid method is generally used for screening, with negative results accepted as such, but positive results require cultural confirmation by the appropriate official method. Isolate subtyping with a method such as serotyping or genetic fingerprinting may be used for tracking and troubleshooting purposes.

TABLE 5. Examples of locations and situations in facilities that can serve as potential sources for spread of *Salmonella*

Process area

- Aspirator line
- Dust collection system
- Filter sock
- Air conveyance system, e.g., rotary air lock, cyclone, air locks, duct work, pneumatic conveyance system
- Inside a pump that was disassembled
- Inside an air duct
- Exposed insulation
- Eroded flooring
- Space between walls
- Poorly sealed wall/floor junction
- Leaky roof
- Leaky drain pipe
- Conveyor
- Bucket elevator
- Fork lift
- Employees
- Fans
- Cat walks
- Central and/or portable vacuums
- Maintenance tools
- Floor scrubber
- Floor squeegee
- Mop head
- Drain
- Insects, rodents, and other pests

Outside of process area

- Fire exit, for example, used by construction crew to enter and exit the facility
- Entrance to employee locker room
- Pathway to trash compactor
- Receiving dock
- Insect light traps
- Areas where employees may congregate, such as a designated smoking area

* This list is by no means all-inclusive.

TABLE 6. An example of intensified environmental monitoring and control in response to special plant events

Plant events include construction, new equipment installation in the processing areas, or other events that may affect the Primary *Salmonella* Control Area. Plant traffic controls, room air pressure, sanitation activities, etc. should be assessed during construction activities. Intensified environmental control procedures and action steps may be required, including:

- ∞ Reinforce GMP practices and traffic patterns with outside contractors.
- ∞ Set up temporary control barriers within the plant as applicable.
- ∞ Increase cleaning frequency of adjacent areas during construction, after equipment installation, and after major repairs are completed.
- ∞ Perform sampling and testing for *Salmonella* in the construction areas and adjacent areas during construction.
- ∞ Increase environmental monitoring (frequency and/or number of samples) after construction, equipment installation, or major repairs are completed. The sampling sites and frequency should be determined based on a team evaluation of the following: plant location of construction activities; type of construction (e.g., installation, demolition, material removal); duration of construction activities; types of environmental controls implemented, etc.

  – Compositing environmental samples (combining multiple sponges or swabs into one pre-enrichment) or pooling (combining 2–5 post-enrichment samples into one test sample to be run on a rapid method) is generally not recommended. A positive finding on a composited sample cannot identify the specific location of the positive and results in broader, less focused corrective actions. However, there may be some situations where compositing may be appropriate, e.g., samples taken from multiple drains in the same processing area, where it is less important to pinpoint the site. If a "pooled" sample comes up positive, the individual enrichments that made up the pooled sample can be immediately retested separately to pinpoint the positive sample(s). However, this process adds delay in determining the location of a positive, compared to testing samples individually. The ability to composite or pool samples is method dependent and must be validated. Implications of compositing or pooling should be carefully considered.

❑ Corrective actions must be taken when *Salmonella* is detected in an environmental monitoring or finished product sample. In most cases, corrective actions are triggered by presumptive *Salmonella* test results, since waiting for the final confirmation could take up to a week.

  – If a positive is found in any of the four sampling zones, the site should be examined and potential causes investigated. It may be advantageous to have a pre-assigned team to assist in the investigation and to help direct corrective actions.
  – Corrective actions to be taken should be based on an assessment of the potential for finished product contamination, given the location of the positive site in the environment. (A positive in Zone 2, 3, or 4 (non-PCS) does not automatically implicate finished product.)
  – Corrective actions should include appropriate procedures, such as those described in Table 4, and be accompanied by re-sampling of the initial positive and adjacent areas.
  – All corrective actions taken, including re-sampling results, should be documented.

SUMMARY AND KNOWLEDGE GAPS

Several significant outbreaks of foodborne salmonellosis have been linked to products produced in low-moisture-food manufacturing environments. The control of *Salmonella* in these environments is challenging and highly specialized. Validation is complicated by the increased heat resistance of *Salmonella* at low a_ws. Stringent environmental monitoring regimens are essential to verify control of *Salmonella* in the facility. The guidance presented in this paper and its two companion papers has been developed on the basis of a synthesis of industry practices and programs, as well as information from the literature. Application of the guidance, in terms of control elements and stringency of control, will depend on the product and process, including the intended use of the product.

Knowledge gaps remain to be filled. The lack of adequate *Salmonella* inactivation data in specific products at various water activity levels has hindered industry's ability to evaluate the adequacy of certain processes (such as baking of peanut butter cookies) in the event that an ingredient was found contaminated with *Salmonella*. For example, in response to the 2008–2009 *Salmonella* Typhimurium outbreak linked in part to peanut butter, many peanut butter-containing products were recalled because there was little basis for the companies involved to evaluate the adequacy of the lethality of the specific processes. Although heat resistance data for *Salmonella* in peanut butter were available, data on inactivation of *Salmonella* in peanut butter-containing cookie dough had not been published. The application of the data based on peanut butter was not appropriate to determine whether the baking process was adequate to eliminate the level of *Salmonella* expected in the contaminated ingredient (i.e., peanut butter).

Development and validation of additional dry cleaning methods is needed to

help minimize the risk of post processing contamination. Further work is needed to develop practical molecular subtyping tools with high discriminatory power to facilitate more effective environmental monitoring and *Salmonella* control. Molecular subtyping tools will help establish links between isolates (e.g., from ingredients and processing environment) and differentiate transient versus resident strains in the environment *(30)*. Conducting surveys to determine the prevalence and concentration of *Salmonella* in widely used raw ingredients, in combination with using such data to conduct risk assessments for various products or product/process combinations, will generate further scientific support for the appropriate log reduction, and will facilitate the determination and evaluation of effective control measures and risk mitigation strategies. To this end, more research on dose-response is needed to improve risk assessments, because available *Salmonella* dose-response models, such as the one derived from human studies *(20, 24)* in which a cocktail of serotypes in buffer was fed to healthy adults, may not be representative of the susceptibility of the general population or the risk from low-moisture products. As indicated previously, in some instances, illnesses occurred upon consumption of low-moisture products contaminated at levels < 1 CFU/g, depending on the host, the product, and the *Salmonella* strain.

Continuing research to enhance knowledge in areas such as molecular subtyping tools, more efficient environmental sampling, rapid detection, effective thermal and non-thermal *Salmonella* inactivation processes, and the determination of the appropriate level of *Salmonella* reduction in various low-moisture products, coupled with sharing common industry practices, will enable industry to more efficiently and effectively reduce the risk of *Salmonella* contamination in low-moisture products.

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