Environmental Monitoring Programs in Pet Food Facilities

A EUROFINS WHITE PAPER

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An awareness guide focused on finding pathogens and indicator organisms in the pet food environment and the importance of effective cleaning and sanitation practices.

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What Is the Goal?

Primary	to find pathogens in the environment before they contaminate product
Secondary	to find spoilage microorganisms in the environment before they affect product
Tertiary	to assess effectiveness of cleaning, sanitation, and employee hygiene practices

How Do You Achieve Salmonella and Listeria Monocytogenes Control?

- Thorough cleaning and sanitation
- Traffic control of personnel and equipment
- Separation of raw and extruded product
- Air and dust control
- Water control

Where to Test?

A well-designed environmental monitoring program will include samples from various areas throughout your production processes. The simplest way to organize your sampling program is by zones, as outlined on the next two pages. Identify multiple sampling sites from each zone (based on your specific facility design and processes) before you begin taking samples. If the final number of sampling sites in a zone exceeds the recommended number of samples, you can rotate sites at each sampling interval to increase coverage.

For example, if you have identified 60 potential sampling sites in Zone 2, randomly select 15 sites per week, making sure that each site is sampled at least once per month. This system will help you stretch your testing budget while making sure you sample all sites needed to maintain program effectiveness. Keep a detailed sampling site log and facility map that details locations of sampling sites. The log should contain details on how to collect samples from difficult to access areas (sites under processing equipment, sites within equipment, overhead fixtures, etc.).

Note that the number of sampling sites and number of samples will vary with the type of product and nature of processing scheme. Higher risk products processed in a large complex facility will likely need a greater number of samples and a greater sampling frequency than lower risk products processing in a smaller, more simple process facility.

Zone 1: Direct Product Contact Surfaces

Surfaces where finished product is exposed to the environment before final package closure.

Locations

Monitoring should encompass the entire facility, such as tables, conveyor belts, buckets, fillers, hoppers, utensils, employee hands and gloves, items and surfaces directly over or in close proximity to direct food contact surfaces such as light fixtures and piping, compressed air lines, and water filters.

Tests

Pathogens (*Salmonella* and *Listeria monocytogenes*) and indicator bacteria (*Listeria* species, aerobic plate count, coliform count, total Enterobacteriaceae count, lactic acid bacteria count).

Frequency of Testing

Weekly

Minimum Number of Samples

Depends on line complexity

Program Validation

Necessary if only using indicator organisms in the routine program. Validation includes periodic testing for pathogens of concern.

Product Disposition

All product produced on the line tested should be held until final results are received when testing any Zone 1 sites for pathogens.

ZONE 2: Non-Product Contact Surfaces Close to Zone 1 Surfaces

Locations

Equipment frames, drip shields and pans, control panels and buttons, overhead fixtures and piping not directly over or in close proximity to food contact surfaces, computer screens, maintenance tools.

Tests

Salmonella and indicator bacteria (*Listeria* species, aerobic plate count, coliform count, total Enterobacteriaceae count, lactic acid bacteria count)

Frequency of Testing

Weekly

Minimum Number of Samples

10 to 15

Zone 3: Non-Product Contact Surfaces in Open Processing Area

Locations
Floors, walls, ceilings, drains, hoses, cleaning equipment including brooms and brushes, air handling units, condensate drip pans, carts, pallets, forklifts, trash cans, foot baths, sink area including soap and towel dispensers

Tests
Salmonella and indicator bacteria (Listeria species, aerobic plate count, coliform count, total Enterobacteriaceae count, lactic acid bacteria count)

Frequency of Testing
Weekly

Minimum Number of Samples
10 to 15

ZONE 4: Support Facilities Not in Open Processing Area

Locations
Bathrooms, locker rooms, cafeteria and break rooms, office rooms, hallways, warehouse, loading docks, maintenance shop, storage areas

Tests
Salmonella and indicator bacteria (Listeria species, aerobic plate count, coliform count, total Enterobacteriaceae count, lactic acid bacteria count)

Frequency of Testing
Monthly

Minimum Number of Samples
10 to 15

Aseptic Collection of Environment Samples

Collecting environment samples in an aseptic manner is critical to ensuring the quality of the testing results. If the person collecting the samples contaminates the specimen, the laboratory result will not accurately reflect the condition of your manufacturing environment. There are multiple commercial sampling tools available for use. This instruction addresses three of the most common types.

Cellulose Sponge in Bag

A small cellulose sponge (about 1" x 2") is pre-moistened with a transport buffer in a sealed bag. Some vendors attach a pair of sterile gloves to the sponge bag.

1. Wash your hands up to the forearm. Use clean disposable towels to dry your hands.
2. Using an indelible ink pen (such as a Sharpie) label the sample bags on lower frosted portion with the swabbing location identification or code.
3. Before donning gloves, open the sponge bag and using the tabs on either side, widen the opening.
4. Using one hand (may be ungloved), working from the outside of the bag, carefully move the sponge up to the top of the bag. Squeeze out excess buffer so that the sponge is moist but not dripping.
5. Using the gloved hand, remove the sponge from the bag, being sure not touch the outside of the bag.
6. Thoroughly swab the area to be sampled using even but forceful back and forth motions. Swab an area of about 10 x 10 cm (4 x 4 in).
7. Carefully replace the sponge into the bag without touching the edges of the opening.
8. Close the bag by rolling the top down 3 twists and folding in the tabs. Store the sponge at refrigerated temperature until sent to the lab for analysis.
9. Discard gloves and repeat for each sponge sample collected.
10. Send to the lab via overnight delivery in a cooler chilled with ice packs.

Sponge with Handle ("Spongesickle" or "Stick-Sponge")

A small cellulose sponge is attached to the end of a long plastic handle; the whole unit is contained in a sealed plastic bag containing a small amount of transport buffer.

1. Wash your hands up to the forearm. Use clean disposable towels to dry your hands.
2. Using an indelible ink pen (such as a Sharpie) label the sample bags on lower frosted portion with the swabbing location identification or code.
3. Before donning gloves, open the sponge bag and using the tabs on either side, widen the opening. Glove use is optional if hands remain in sanitary condition between samples. Open the bag, and working from the outside of the bag, carefully move the sponge up so that the handle protrudes from the bag.
4. Using one hand, grasp the handle and remove the sponge so that it does not touch the edge of the opening.
5. Thoroughly swab the area to be sampled using even but forceful back and forth motions. Swab an area of about 10 x10 cm (4 x 4 in).

6. Carefully replace the sponge back into the bag only far enough so that you can grasp the sponge in one hand from the outside of the bag. Using your other hand, bend the sponge handle 90 degrees back and forth three times then twist handle so that it separates from the sponge. Do not insert your hand into the bag.
7. Remove the handle and allow the sponge to drop into the bag. Discard handle.
8. Close the bag by rolling the top down 3 twists and folding in the tabs. Store the sponge at refrigerated temperature until sent to the lab for analysis.
9. Repeat for each sponge sample collected.
10. Send to the lab via overnight delivery in a cooler chilled with ice packs.

Swab ("Q-tip" style)

1. Wash your hands up to the forearm. Use clean disposable towels to dry your hands.
2. Using an indelible ink pen (such as a Sharpie), label the swab container lower frosted portion with the swabbing location identification or code. Use of labeling tape may be helpful due to small size of tubes.
3. Some swabs are premoistened in the carrying tube while others have transport buffer in a small reservoir at the top of the swab handle. Such swabs can be used either dry or wet by breaking the reservoir tab and squeezing buffer into the tube before use. Carefully twist open the swab and remove from the carrying tube using a gloved hand. Glove use is optional if hands remain in sanitary condition between samples.
4. Thoroughly swab the area to be sampled using even but forceful back and forth motions. Swabs are useful to collect samples from nooks and crannies where sponge devices will not penetrate. Carefully replace the swab into the tube without touching the edges of the opening.
5. Close the tube by tightly twisting the swab. Store the swab at refrigerated temperature until sent to the lab for analysis.
6. Send to the lab via overnight delivery in a cooler chilled with ice packs.

Sponge/Swab Transport Buffers

The most common transport buffers contain various agents that act to neutralize common sanitizers that might be present in the collected samples. Any residual sanitizer could have bactericidal and/or bacteriostatic activity, which could negatively impact the ability to count or recover the target organisms by enrichment and/or by direct impact on downstream detection methods. The three most common neutralizing buffers are Letheen broth, Neutralizing buffer, and D/E broth. Their neutralizing components and sanitizers inactivated are listed below.

Letheen broth

- Lecithin – quaternary ammonium compounds
- Polysorbate 80 – substituted phenolics
- Both together – ethanol

Neutralizing buffer

- Sodium thiosulfate – iodine and chlorine
- Aryl sulfonate complex – quaternary ammonium compounds

D/E broth

- Sodium thioglycolate – mercurials
- Sodium thiosulfate – iodine and chlorine
- Sodium bisulfite – aldehydes
- Polysorbate 80 – substituted phenolics
- Lecithin – quaternary ammonium compounds

Typical Sanitizing Agents	Letheen Broth	Neutralizing Buffer	D/E Broth
Quaternary Ammonium Compounds (Quats)	Yes	Yes	Yes
Iodine Preperations	Somewhat	Yes	Yes
Chlorine Preperations	Somewhat	Yes	Yes
Peroxide or Peroxyacetic Acid	Yes	Yes	Yes
Phenolics	Yes	No	Yes
Glutaraldehyde	No	No	Yes

Due to differences in the composition of these three common transport buffers not all are compatible with all detection methodologies. For example D/E broth is purple and can affect detection methods that require visual observation of a color change, such as Petrifilm or others. Additionally, the neutralizing agents themselves can have negative effects on some detection technologies. It is important to select the transport buffer that gives the broadest protection against the sanitizers most commonly used in a production process. Potential impacts on the selected detection method may need to be considered particularly if a change in transport buffer is implemented.

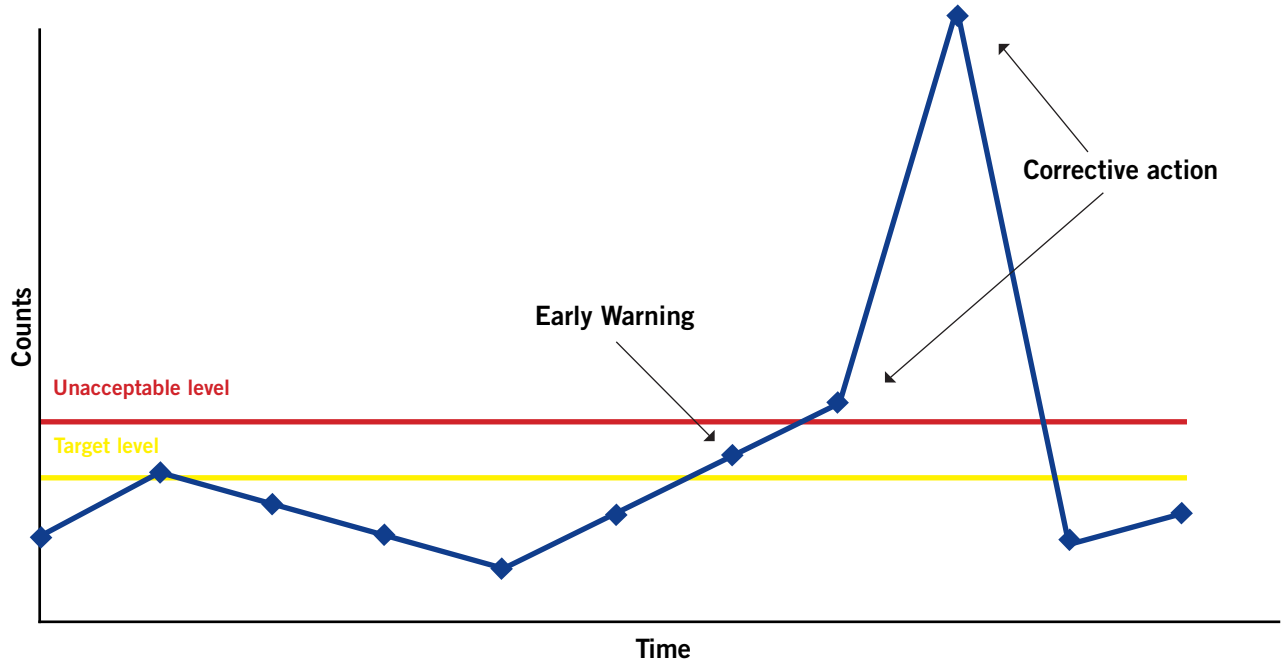
Record Keeping

- Sample log
- Use log book or spreadsheet
- Date and time of sampling
- Name of person collecting sample
- Sample locations
- Date submitted to laboratory
- Results
- Corrective actions if needed

Establish a Baseline

Collect preliminary indicator counts for each location and determine a population level for each indicator to serve as a target level. For each indicator, also establish a population level that is unacceptable.

Establish a Baseline and Trend Tracking



Example Performance Targets

Indicator	Action Levels	Before Sanitation	After Sanitation
Aerobic Plate Count	Target	<100	<10
	Acceptable	<500	<100
	Unacceptable	>500	>100
Coliforms	Target	<10	<10
	Acceptable	<100	<50
	Unacceptable	>100	>50
Total Enterobacteriaceae	Target	<10	<10
	Acceptable	<100	<50
	Unacceptable	>100	>50
Lactic Acid Bacteria	Target	<10	<10
	Acceptable	<100	<50
	Unacceptable	>100	>50

What To Do When Elevated Indicator Counts Are Found?

1. Break down and inspect equipment
2. Thoroughly clean and sanitize all equipment, surfaces, and tools in area
3. Re-sample areas where high counts found
4. Re-clean, re-sanitize, and re-sample as needed
5. If high counts persist, implement corrective actions

Corrective Actions If *Salmonella/Listeria Monocytogenes* Found

- Stop processing in affected area
- Limit access to area
- Break down and inspect equipment in area
- Re swab positive area and surroundings to determine whether contamination is localized or spread
- Clean and sanitize all equipment, surfaces, and tools in area
- Conduct pre-operational inspection and re swab
- Do not restart operations until all tests are negative
- Document corrective actions and consider SOP to prevent recurrence
- Increase frequency of sampling from weekly to daily
- After three consecutive days of negative results normal sampling may resume
- If problem persists, consider removal of contaminated equipment and replace or redesign

Routine Preventive Controls

- Control sources of water and air
- Repair structural damage and eliminate cracks and crevices that can harbor microbes
- Review and monitor GMPs
- Review and monitor SSOPs
- Audit production and maintenance practices
- Reinforce proper employee hygiene practices

Testing needs vary widely across facilities; connect with Eurofins today to learn how we can serve your unique food quality and safety needs: Info@EurofinsUS.com