

Exercise 1: Case Study – Contamination by *Listeria monocytogenes*

A smoked salmon factory detects *Listeria monocytogenes* on sliced fillets. Analysis shows that the contamination is localized on slicer N^o. 2 and the associated packaging table. The bacterium is not detected upstream.

The three categories are: biologic, chemical and physic.

And the categories of potential sources for this contamination : Primary (or original) source, reservoir, and vector.

In this case: The *Listeria monocytogenes* found on the equipment is considered a reservoir. It is an environment where the bacteria has established itself, persists, and from which it can contaminate food products. The primary source (e.g., the initial introduction into the factory environment) remains to be identified.

2. Propose a list of at least five hypotheses explaining how the equipment could have been contaminated (think of the 5M's: Environment, Equipment, Manpower, Methods, Materials).

Environment (Milieu): Aerosols or water droplets from a contaminated floor drain or cleaning system near the equipment.

Equipment (Matériel): A defective or poorly designed part of the slicer (e.g., a hollow frame, plastic guides) that is difficult to clean and has allowed biofilm formation.

Manpower (Main d'œuvre): An operator carrying the bacteria (on shoes, gloves, or sleeves) from a contaminated zone (e.g., raw material area, maintenance workshop) to slicer N^o. 2.

Methods (Méthode): Ineffective or inadequately applied cleaning and disinfection procedure for slicer N^o. 2, allowing progressive buildup of residue and bacterial growth.

Materials (Matières): Contamination introduced via a raw material (e.g., a salmon belly flap heavily contaminated with *Listeria*) that was processed on this specific slicer, seeding the equipment before it was eliminated by the smoking step for subsequent products.

3. Isolation and Quarantine: Immediately stop production on slicer N^o. 2. Physically isolate the equipment and the associated packaging table. Block all products that have passed through this equipment since the last negative microbiological control or since the last effective cleaning.

Product Hold: Place the affected production lots on hold, pending a risk assessment and potential disposal or recall.

Intensified Cleaning and Disinfection (C&D): Perform an emergency, reinforced C&D of the contaminated equipment and its immediate surroundings, following a pre-defined "outbreak" procedure.

Reinforced Sampling: After C&D, conduct environmental swabbing (equipment, surfaces) and possibly product sampling to verify the effectiveness of the intervention before any restart authorization.

4. Protocol for Enhanced C&D of Slicer N°. 2:

Preparation & Dismantling: Disconnect the equipment. Dismantle it as much as possible (blade, guards, conveyors, trays) according to the technical diagram to expose all surfaces.

Dry Removal & Pre-rinsing: Remove large debris. Perform a copious pre-rinse with potable water (approx. 45°C) to remove organic matter.

Washing & Scrubbing: Apply an alkaline detergent (foam or gel) suitable for fighting biofilms. Allow sufficient contact time. Scrub all accessible and dismantled parts meticulously with dedicated brushes. Pay special attention to crevices, joints, and hollow parts.

Rinsing: Rinse thoroughly with potable water to remove all detergent and suspended soil.

Disinfection: Apply a broad-spectrum disinfectant effective against *Listeria* (e.g., peracetic acid, quaternary ammonium). Respect the recommended concentration and contact time (often >5 minutes). For critical parts, consider thermal disinfection with hot water (>82°C) if the equipment allows.

Final Rinsing & Drying: Perform a final rinse with potable water if the disinfectant requires it (check manufacturer's instructions). Allow all parts to air dry completely before reassembly to prevent microbial regrowth.

Control Methods:

- Visual Control: Verify the absence of visible residue or grease on all parts after rinsing and before disinfection.
- ATP Bioluminescence Testing: Perform rapid testing on key surfaces after C&D to verify the removal of organic matter (set a very strict pass threshold, e.g., <50 RLU).
- Microbiological Verification: After C&D and drying, take environmental swabs (especially from high-risk zones) for analysis of *Listeria* spp. The equipment can only be released for production after obtaining negative results.

Exercise 2: Multiple Choice Questionnaire (MCQ)

c) A glass fragment

b) Mechanical Action

c) An acid detergent

c) The amount of ATP, an indicator of the presence of organic matter

Exercise 3 : Problem of dilution

1. Calculate the volume of concentrated product (in L) required.

The formula for volume/volume percentage concentration is:

$(\text{Volume of solute} / \text{Volume of solution}) * 100 = \% \text{ concentration}$

Therefore:

$\text{Volume of solute} = (\% \text{ concentration} * \text{Volume of solution}) / 100$

$\text{Volume of solute} = (0.5 * 50) / 100 = 25 / 100 = 0.25$

Answer: 0.25 liters (or 250 mL) of concentrated product is required.

2. What volume of water (in L) will you need to add?

The final volume of the solution is 50 liters. This volume includes both the concentrated product and the water.

$\text{Volume of water} = \text{Final volume} - \text{Volume of concentrate}$

$\text{Volume of water} = 50 \text{ L} - 0.25 \text{ L} = 49.75 \text{ L}$

Answer: 49.75 liters of water must be added.

3. An operator uses only half the dose. What are the potential consequences?

Using only half the dose (0.125 L instead of 0.25 L) results in a solution at 0.25% concentration instead of the required 0.5%.

Potential consequences:

Reduced Efficacy: The disinfectant may be ineffective or have insufficient biocidal power against target microorganisms (like *Listeria*). This increases the risk of microbial survival and biofilm formation.

Development of Resistance: Prolonged use of sub-lethal concentrations can promote the development of microbial resistance to the disinfectant.

False Sense of Security: Personnel believe the area is properly sanitized, leading to a higher risk of product contamination.

Non-Compliance: The procedure is not followed, which is a critical deviation in a food safety management system (e.g., HACCP).

4. How would you check the actual concentration of the prepared solution in the field?

Answer: Several methods can be used for on-site verification:

Refractometer: If the concentrated product significantly alters the refractive index of water, a quick measurement can indicate if the dilution is approximately correct. This requires prior calibration for the specific product.

Test Strips: Specific chemical test strips (if available for the active ingredient in the disinfectant, e.g., for peracetic acid, quaternary ammonium compounds) can provide a semi-quantitative concentration reading by color comparison.

Titration Kits: Portable titration kits allow for a more precise measurement of the active ingredient's concentration. An operator adds a reagent to a sample of the solution drop by drop until a color change occurs, indicating the concentration.

Conductivity Meter: For some ionic disinfectants (e.g., hypochlorite), the conductivity of the solution is proportional to its concentration. This method also requires a calibration curve for the specific product.

The most suitable method depends on the nature of the disinfectant and the resources available in the factory. This verification is a key step to ensure the effectiveness of the sanitation procedure.

Exercise 4: Development of a Cleaning Procedure

Standard Operating Procedure (SOP): Cleaning and Disinfection of Vegetable Preparation Cutting Table

1. Identification

Area/Equipment: Stainless steel cutting table (Station: Veg-Prep-01).

SOP Reference: SOP-C&D-VEG01

Effective Date.

2. Purpose

To ensure the cutting table is cleaned and disinfected to a hygienic standard that prevents cross-contamination and ensures product safety.

3. Scope

This procedure applies to all personnel responsible for cleaning this specific cutting table.

4. Frequency

Between product batches: Cleaning and disinfection (Rinse, Clean, Disinfect).

At the end of each production day: Full cleaning, disinfection, and drying.

Before production start-up (after a shutdown): Verification and, if necessary, re-disinfection.

5. Personal Protective Equipment (PPE)

Heavy-duty rubber gloves

Protective apron

Safety goggles (if using pressurized spray or splashing risk)

Closed, non-slip shoes

6. Materials and Products

Materials: Dedicated color-coded (e.g., green for veg area) brushes and scrapers, non-abrasive scouring pads, dedicated buckets, low-pressure hose with spray gun, clean lint-free cloths.

Detergent: Alkaline detergent, suitable for food contact surfaces. Concentration: 2-3% as per manufacturer's instructions (e.g., 20-30 mL per liter of water at 45-55°C).

Disinfectant: Food-grade, broad-spectrum disinfectant (e.g., quaternary ammonium compound, peracetic acid). Concentration: As per manufacturer's instructions for food contact surfaces (e.g., 0.5% v/v with a 5-minute contact time).

Note: Rinse water must be potable.

7. Procedure Steps

Phase 1: Preparation (2 min)

- 1.1. Remove all vegetables, tools, and waste from the table surface and surrounding area.
- 1.2. Dismantle removable parts (e.g., side guards, drip trays) if applicable.
- 1.3. Pre-rinse the table and parts with potable water (~45°C) to remove loose soil.

Phase 2: Washing/Cleaning (5 min)

- 2.1. Prepare the detergent solution in a dedicated bucket at the correct temperature and concentration.
- 2.2. Apply the solution generously to the entire table surface, legs, and underside edges.
- 2.3. Scrub thoroughly with the dedicated brush, paying special attention to corners, welds, and any surface imperfections. Use a scraper for any adhered residues.
- 2.4. Clean all removable parts in the detergent solution.
- 2.5. Rinse thoroughly with potable water until all detergent foam is removed and the rinse water runs clear.

Phase 3: Disinfection (5 min contact time)

- 3.1. Prepare the disinfectant solution in a second dedicated, clearly labeled bucket/sprayer at the correct concentration.
- 3.2. Apply the disinfectant evenly over all cleaned surfaces using a sprayer or a cloth soaked in the solution, ensuring complete coverage.
- 3.3. Allow the disinfectant to remain wet on the surface for the full manufacturer-specified contact time (e.g., 5 minutes). Do not rinse.

Phase 4: Final Rinse & Drying (3 min)

- 4.1. Only if required by the disinfectant manufacturer's instructions: Perform a final rinse with potable water after the contact time. (If no rinse is required, skip to 4.2).

4.2. Wipe the surface with a clean, dry, lint-free cloth or allow to air dry completely in a ventilated area. Assembled equipment must be completely dry before use.

Phase 5: Storage & Waste Disposal (2 min)

5.1. Store all cleaned and dried removable parts in a clean, designated area.

5.2. Empty, clean, and store buckets and brushes in their designated sanitizing station.

5.3. Dispose of waste water and used cloths according to hygiene waste procedures

8. Control Points

CP1 (Visual): Absence of visible soil, grease, or vegetable residues after rinsing.

CP2 (Visual/Chemical): Correct preparation and labeling of detergent/disinfectant solutions (concentration, temperature).

CP3 (Temporal): Respect of the disinfectant contact time.

CP4 (Final State): Surface is completely dry before next use.

Verification Plan: Validation by the hygiene manager, signed traceability sheet, comparative monthly ATP control.

Documents to be filled in:

Cleaning Checklist (SOP-CLD-VEG01-F01): To be signed for each cleaning cycle.

ATP Control Log: To record RLU values, date, time, and operator.

Microbiological Swab Report: Official lab report.

Non-Conformity Report (NCR): For any deviation (high ATP, visual failure, incorrect concentration).

Exercise 5:

1. Daily cleaning of a stainless steel tank showing "milk stone" deposits.

Choice: Acid Detergent

Justification:

"Milk stone" is not actually a stone; it is a mineral deposit primarily composed of calcium and magnesium phosphates and proteins. These are **inorganic** (mineral) deposits. Alkaline detergents are excellent for removing organic soils (fats, proteins) but are less effective on mineral scales. Acid detergents (such as those containing phosphoric, nitric, or sulfamic acid) are specifically designed to dissolve these hard water scales and mineral salts by a chemical reaction (chelation or pH neutralization).

2. Degreasing at the end of the day of a heavily soiled industrial fryer.

Choice: Alkaline Detergent

Justification:

Cooking oil and grease are **organic** soils. High-temperature cooking causes fats to polymerize, forming a tough, baked-on layer. Alkaline detergents work by **saponification**: they react with fats to convert them into soap (which is water-soluble) and glycerol. A heavy-duty alkaline detergent (often chlorinated or with high pH) is required to break down these heavy, carbonized grease deposits effectively.

3. Manual washing of fragile, lightly soiled plastic utensils.

Choice: Neutral Detergent

Justification:

Plastic can be sensitive to high temperatures and aggressive chemicals, which might cause it to warp, crack, or become brittle over time. Since the soil load is light, there is no need for aggressive chemistry. Neutral detergents (pH around 7) are safe for most materials, gentle on the skin of the person washing them manually, and provide sufficient cleaning power for light soil through mechanical action (scrubbing) and wetting agents (surfactants).

4. Cleaning a conveyor belt with dried protein residues.

Choice: Alkaline Detergent or Enzymatic Detergent

Justification:

Proteins are **organic** soils. When proteins are heated or dried, they can denature and adhere strongly to surfaces. Alkaline detergents are effective because high pH breaks the peptide bonds in proteins (hydrolysis), making them soluble and easier to rinse away.

However, in the specific context of a conveyor belt (which may be made of plastic or rubber that could be sensitive to high temperatures/chemicals), an **Enzymatic Detergent** might be the "most suitable" choice if we consider precision cleaning:

- **Enzymes** (specifically proteases) are biological catalysts that "cut" protein chains into smaller, water-soluble pieces at neutral or mild temperatures.
- They work at lower temperatures and pH levels, which preserves the conveyor belt material, saves energy, and is safer for the environment and staff. They are highly specific to protein residues.

Exercise 6:

Analysis of the Situation

The Facts:

1. **Product:** Yogurt (high in protein and fat).

2. **Location:** Dosing head of machine no. 4 (a difficult area to clean, often with complex geometry: nozzles, pistons, gaskets).
3. **Data:** ATP measurements are non-compliant (high Relative Light Units = presence of organic residue).
4. **Paradox:** Visual inspection is satisfactory (the surface looks clean) and the protocol is theoretically respected.

Interpretation:

The ATP meter detects biological residue invisible to the naked eye (microorganisms or food residue). Since the visual is clean but the ATP is high, the contamination is either microscopic (biofilm) or located in a place that is not visible (internal parts of the dosing head).

Hypotheses to Explain the Problem

Here are the main possible hypotheses, ranked by likelihood:

1. **Inaccessible Design (Critical Point):** The dosing head has "dead zones," micro-crevices, or seals that retain yogurt. The cleaning solution does not achieve sufficient turbulence to dislodge the residue in these blind spots.
2. **Ineffective Pre-rinsing:** Proteins (in yogurt) denature and stick when heated. If the pre-rinse water is too hot (above 60°C), the proteins "cook" onto the surface before the detergent has a chance to act, forming a film that is resistant to cleaning.
3. **Chemical or Mechanical Problem:**
 - The detergent concentration is correct in the tank, but the dosing head is not being fully submerged or sprayed effectively.
 - The cleaning temperature is incorrect for the detergent used.
 - The cleaning time is insufficient for this specific piece of equipment.
4. **Biofilm Formation:** A community of bacteria has established itself in a hard-to-reach area. They secrete a protective matrix (glycocalyx) that resists standard detergents and holds onto organic matter, generating the ATP signal.
5. **Soil Return:** During the rinse, water carries soil from a dirty upstream pipe back into the now-clean dosing head.

Proposed Action Plan (Investigation)

To find the root cause, you must move from observation to investigation. Here is a step-by-step action plan:

Step 1: On-site Observation (Go & See)

- Do not just read the protocol; watch the operator perform the cleaning.
- **Verify the parameters in real-time:** Is the temperature of the pre-rinse water actually correct? Is the flow rate/pressure strong enough to reach the top of the dosing head?

- **Check the dismantling:** Is the operator completely dismantling the dosing head (nozzles, pistons, seals) as required, or are they cleaning it assembled?

Step 2: Detailed Inspection

- **Dismantle completely** the dosing head.
- Inspect the seals and gaskets: are they swollen, cracked, or encrusted with residue?
- Check the internal surfaces of the nozzles by running a cotton swab inside them, then test that swab with the ATP meter. This will tell you if the residue is internal.

Step 3: Challenge the Chemistry

- Check the detergent's expiry date and the concentration in the tank (using a titration kit, not just the conductivity, as yogurt can alter conductivity readings).
- **Hypothesis test:** Perform a manual soak of the small parts in a highly concentrated enzymatic or alkaline detergent. Rinse and re-test with ATP. If they become clean, the CIP (Clean-in-Place) parameters are insufficient.

Step 4: Analyze the Rinse Water

- Test the final rinse water with ATP before it touches the equipment. If the water itself is contaminated, it is recontaminating the equipment (biofilm in the pipes).

Step 5: Modify the Protocol (Corrective Test)

- If the investigation points to protein denaturation: change the pre-rinse to **cold/lukewarm water** first to remove the organic matter before it "cooks."
- If the investigation points to a mechanical dead zone: increase the flow rate, add a specific cleaning cycle for the dosing head (e.g., pulsing the dosing head during cleaning to create turbulence in the cylinders), or change the dismantling frequency.