



THE DAIRY PRACTICES COUNCIL®

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# GUIDELINE FOR TROUBLESHOOTING MICROBIAL DEFECTS IN DAIRY PROCESSING PLANTS: PRODUCT-LINE SAMPLING AND HYGIENE MONITORING

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## Guideline Preparation and Review Process

Guideline development within Dairy Practices Council (DPC) is unique and requires several levels of peer review. The first step in the process of guideline development starts with a Task Force subcommittee comprised of individuals from industry, regulatory and education interested in and knowledgeable about the subject to be addressed. Drafts, referred to as ‘white copies,’ are circulated until all members are satisfied with the text. The final white copy may then be distributed to the entire Task Force, DPC Executive Vice President and whoever the Task Force Director feels would add to the strength of the review. Following final white copy review and correction, the next step in the process requires a yellow cover draft that is circulated to the member Regulatory Agency representatives that are referred to as “Key Sanitarians.” The Key Sanitarians may suggest changes and insert footnotes if their state standards and regulations differ from the text. After final review and editing the guideline is distributed in the distinctive DPC green cover to people worldwide. These guidelines represent the state of the knowledge at the time they are written.

## Contact Statement

This guideline was developed by contributors who are of experienced individuals in a related field(s). The acknowledged persons are included with their professional affiliations and may be contacted via DPC Officer(s) and/or Task Force Director(s) for questions or concerns.

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# INTRODUCTION

Quality defects in dairy products are most often the result of microbial contamination, growth, and spoilage. In most cases, product contamination is the result of insufficient cleaning and sanitation of the processing equipment and plant environment. However, product contamination occurs even when it appears that a well-designed sanitation and quality control program is in place. When this happens, it is often the responsibility of quality control personnel to determine the cause and source of contamination. Though the problem usually becomes evident in the finished product, microbiological testing of the finished product alone, in most cases, does not provide sufficient information to determine the source(s) of microbial contamination; more extensive testing is usually required. In this regard, testing product at different steps during processing to isolate contamination sources (product line-sampling) and evaluating the cleanliness of food contact surfaces (hygiene monitoring) represent valuable tools for a dairy plant quality control program.

Product line-sampling involves the collection and analysis of samples at successive points in a process in order to determine the source of microbial contamination. In theory, line-samples taken before the source of contamination should be free of contaminants (i.e. spoilage or indicator organisms), while line-samples taken after the contamination source should contain the contaminant. The source of contamination can then be narrowed down to the area between the last sample without the contaminant and the first sample with the contaminant.

While line-sampling procedures evaluate processing conditions by analysis of the product, hygiene monitoring is the direct evaluation of food product contact surfaces for the detection of microorganisms or food soil that might result in the contamination of a product. Surface swabbing and direct contact procedures are common tools used for determining the extent of microbiological contamination of a surface. Rapid hygiene monitoring systems have been commercially developed that detect ATP associated with microorganisms or food soil.

Though valuable tools in a quality control program, line-sampling and hygiene monitoring can only give useful information if appropriate microbiological tests are performed and properly interpreted. When designing a microbiological sampling and testing program, there are several factors that should be considered. These include the types of organisms that cause defects in a product, the level of contamination and the conditions that support their growth. The characteristics of a specific dairy product (i.e. pH, moisture, etc.) along with the processing and handling conditions used for that product may limit the possible sites of product contamination and the types and numbers of microorganisms that are of concern. The sampling sites and the microbiological methods should be selected based on this knowledge and interpreted relative to the product quality.

# DEFINITIONS

**ATP** – Adenosine Triphosphate

**SMEDP** – Standard Methods for the Examination of Dairy Products

**SPC** – Standard Plate Counts

**LPC** – Lab Pasteurization Counts

**CVTA** – Crystal Violet Tetrazolium Agar

**VRBA** – Violet Red Bile Agar



T.T.C. – Triphenyl Tetrazolium Chloride

RLU – Relative Light Units

CFU – Colony Forming Units

## GUIDELINE CONTENT

### **Product Line – Sampling – Pasteurized Fluid Milk**

Pasteurized fluid milk is the major dairy product produced in North America and is often used for the manufacture of other products as well. The quality and shelf life of fluid milk is dependent on the quality of the pre-pasteurized or raw milk and the conditions under which it is processed.

Raw milk is generally the starting point of most processed dairy products. Though the legal limit for commingled raw milk is 300,000 col/ml bacteria, good quality raw milk should have bacteria counts of less than 50,000 col/ml. Raw milk can be a source of thermophilic bacteria that survive pasteurization. Thermophilic bacteria are often responsible for high bacteria counts in pasteurized milk that result in regulatory action. However, thermophilic bacteria are rarely the cause of shortened shelf life which is most commonly associated with contamination of the product after pasteurization.

Often the source of contamination is obvious, making the information provided by line-samples unnecessary. If contamination is found in all milk that comes from one pasteurized tank or that is packaged on a filling machine or that passes through a specific valve, the source can easily be identified. However, if there is no identifiable pattern, line-sampling may provide information that will help solve the problem.

The following is a list of suggested sampling sites in a HTST pasteurization and bottling operation. Many of the sites listed apply to batch pasteurization systems as well. Other sites not listed may be of value. Collection of samples requires the use of sanitized dippers when samples are taken from open tanks or sampling syringes or vacuum tubes when samples are taken from installed sampling ports (described in the next section). The actual sites selected for sampling depends on the nature of the problem and the design of the system. If high bacteria counts in fresh product are a concern, raw milk should be evaluated. If the main concern is shortened shelf life, sampling should concentrate on sites after the heating section of the pasteurizer.

#### Suggested Sampling Sites

##### HTST Pasteurized Fluid Milk

###### Raw Milk

1. Tank truck samples – if high thermophilic counts are found, individual producer samples should be evaluated.
2. Silo or storage tank – from the inlet or outlet line.
3. HTST balance tank.
4. Pipe connecting the raw regeneration section to the heating section of the HTST system.



### Pasteurized Milk

1. Pipeline between regeneration and the cooling section of HTST.
2. HTST discharge – from the pipeline immediately after cooling.
3. Before pasteurized milk tank (PMT) – from the inlet pipe.
4. After pasteurized milk tank – from the outlet pipe.
5. Before valve cluster(s) – from pipeline immediately before.
6. After valve cluster(s) – from pipeline immediately after.
7. Filler bowl – from the pipeline at entry to the filler bowl.
8. Filled package – immediately after the package leaves filler.

Caution: Milk at certain sites in the HTST system is under extreme pressure. Careful consideration should be given to the location of sampling ports at high pressure points.

Generally, only a few of these suggested sampling sites are required to determine a source of contamination, while testing at all sites is labor intensive and generally unnecessary. A less labor-intensive sampling program is to sample after the HTST, before and after the pasteurized milk tank/silo and before the filler bowl. This would isolate the valve cluster though it does not necessarily identify individual valves as sources of contamination.

### Sampling Ports – Fluid Milk

Sampling sites should be selected to isolate different sections of the processing line so that potential sources of contamination can be narrowed down to specific areas. When choosing the location of sampling ports, the ease of access for sampling and dismantling and cleaning of the sampling system should be considered. Sampling ports can be fabricated in-house, or commercial sampling systems can be purchased. Regardless of the type used, it is recommended that, when possible, sampling ports only be installed in sections of pipeline that are removable, such as gasketed elbows. Installation into welded lines is not recommended as these are permanent and can become a source of contamination if not used and maintained.

### Tank Petcocks

Sampling from tank petcocks is not recommended. If used, extreme care must be taken to prevent them from being a source of contamination. Petcocks should be dismantled and thoroughly washed each time the tank is emptied. Before a sample is taken, it is recommended to run product out of the petcock for several seconds. At least two quarts of milk should be flushed through the petcock before sanitizing and collecting a sample. Product that is in the petcock quickly becomes room temperature and will cause high bacteria counts that are not accurate. Flushing the line will allow the warm product to be expelled and actual product from the tank or silo can be collected. The petcock should be thoroughly sanitized prior to sampling with an iodine mixture at the proper concentration or sanitizer used at the facility for at least 30 seconds of contact time. Many times a Whirl-Pak bag is used to sanitize, as it can cover the entire petcock.

### Drilled Sampling Ports

Sampling ports can be fabricated in-house with little expense. The only requirements are rubber stoppers or grommets, hose clamps and a person experienced in drilling stainless steel. The equipment required can be purchased from many local dairy supply companies. Make sure the grommets/ rubber stoppers and hose clamps meet the requirements for sanitary designs.

### Drilled Holes

The diameter of the drilled hole should match the diameter of the grommet used, generally 3/8 to 1/2 inch (see Table 1). Holes should be drilled square with the surface of the pipe,



preferably near an opening so that the hole can be properly smoothed and polished. If elbows are used, the hole should be positioned and drilled on the outer curvature of the pipe.

**Rubber Grommet**

Conventional serum bottle stoppers designed for needle punctures work well as sampling grommets. They are autoclavable and withstand detergents, chlorine, and other dairy chemicals (see Table 1). Caps with recessed holes that may trap milk residues should be avoided. Snap-in caps can be used without hose clamps if the product pressure is low. The diameter of the stem must match the diameter of the drilled hole, while the cap of the stopper should overlap the exterior pipe by at least 1/4 inch.

**Table 1. Drilled Holes and Serum Caps for Sampling Ports**

Hole Diameter	Serum Caps Size
1/2 inch	13 x 20 mm
3/8 inch	10 x 20 mm

**Clamp**

Ring-style hose clamps are used to secure the grommet in place. Clamps should be made of stainless steel or food-safe plastic, have a minimum width of 1/2 inch and be of sufficient length to be tightened around the circumference of the piping.

Drill a hole in the clamp to coincide with the center of the grommet, 2-3 inches from the tightening screw. The hole should be large enough for easy insertion of a syringe needle into the grommet, though it should be smaller than the hole drilled in the pipe. A stainless-steel washer (1/2-inch diameter hole, 3/4-inch overall diameter) can be silver-soldered to the clamp to add extra support, though this may not be necessary.

**Assembly**

Before assembly, sanitize the pipe, the grommet, and the hose clamp. Insert the grommet snugly into the drilled hole, then place the clamp over the pipe so the clamp hole is centered over the grommet cap. Tighten the clamp firmly, so the entire surface of the grommet cap lays flat on the piping. If the grommet protrudes more than 1/8" (3.2 mm) into the product line, it should be trimmed before installation. Sanitize the assembled unit and install into the process line before the system sanitation cycle.

**Commercial Sampling Ports**

Commercial sampling systems are available. These consist of removable (i.e. on an elbow) or welded fittings with sampling membranes secured with a screw-on cap. Aseptic sampling systems use pre-sterilized sample membranes with single track needle channels that limit the number of samples taken per membrane. Membranes are easily replaced.

**Sampling Devices – Fluid Milk**

Selection of sampling devices will depend on the number of samples and types of tests performed. Samples are frequently collected with sterile vacuum tubes or single-use syringes. Syringe sizes range from 10cc to 140cc. Generally, a minimum of 50cc should be taken, especially if a stress test is used to detect low level contamination.



Larger bore needles (18 gauge) may be necessary for sampling viscous products (i.e. whey, whipping cream, eggnog, and ice cream mix). However, care should be taken that the needle bore is not so large that the sample grommet does not self-seal when removed.

Sample Size

With fluid milk, it only takes one psychrotrophic bacteria per container to cause spoilage and reduced shelf life. To detect low level contamination, samples must be subjected to a stress test or a selective incubation period to allow bacteria to increase to detectable levels. The size of the sample will determine the odds of finding contaminants. The larger the sample size, the more likely low-level contamination will be detected (see Table 2).

**Table 2. Influence of Line-Sampling Size on the Odds of Detection**

Line-Sample Size	10mL	50mL	100mL
<b>Contamination Level<sup>1</sup></b>			
1 bacteria per quart	1/100	1/20	1/10
10 bacteria per quart	1/10	1/2	1/1
100 bacteria per quart	1/1	5/1	10/1
1000 bacteria per quart (1 per mL)	10/1	50/1	100/1

Sample Collection – Fluid Milk

Clean and Sanitize the Sampling Port

Before sampling, thoroughly wash and dry the sampling area. Immediately before taking the sample, sanitize the grommet cap with a solution of chlorine (200-250 ppm), iodophor (25 ppm) or 70 % alcohol. Use a spray bottle or a sanitizing hose to thoroughly wet the area with sanitizer. Alcohol swabs, such as those used in the medical field, may also be used. Regardless of the sanitizer used, it should remain in contact with the surface of the sampling port for a minimum of one minute before the needle is inserted.

Assemble and Insert the Needle

Attach a sterile needle to the syringe in a manner that prevents contamination. Remove the needle’s protective cover and immediately insert it into the sampling port. Milk in product pipelines usually has enough pressure to fill the syringe without having to pull the plunger, though gradual pulling may be required.

Caution: Excessive pressure in the line may cause the syringe plunger to be forced out. Keep a hand over the end of the plunger to prevent this.

Fill the sampling device to 95% capacity, then remove the needle from the port. After the needle is removed, immediately return the protective cover to the needle.

It is important to use different entry points for each sample taken and to replace the grommet after at least every ten samples. A mark can be left with a permanent marker where the samples are taken to prevent resampling the same site. This prevents product contamination of any product that has been sitting in a previously used entry point or port.

Clean and Re-Sanitize the Port

<sup>1</sup> Odds of detection, i.e., with 10mL sample, 1 chance in 100 if milk has one psychrotroph per quart.



Thoroughly wash and sanitize the sample port and surrounding area after the sample has been taken.

### Transfer Sample

Sample syringes may be used to hold the sample though it is often an advantage to transfer the milk to a more "user-friendly" sterile container, such as a Whirl-Pak bag or sample vial. The container should be large enough to hold the sample with adequate head space to allow proper mixing. The head space also provides additional oxygen, which promotes the growth of aerobic bacteria, the more common spoilage organisms.

When transferring samples from a syringe, it is best to use aseptic processes. Make sure not to contaminate the syringe or the sterile container. Remove the needle without touching the male end of the needle and dispense the sample into a sterile container by slowly depressing the syringe plunger.

Note: Needles and syringes should be disposed of in a safe and responsible manner after use. Local authorities should be contacted to confirm disposal methods. Glass containers or sampling devices should not be used in the processing area.

### Microbiological Testing – Fluid Milk

Microbiological testing procedures should be conducted in accordance with the most recent edition of *Standard Methods for the Examination of Dairy Products*, (SMEDP). There are additional testing procedures not found in SMEDP that may be useful. Most line-samples can be subjected to the same testing procedures used to evaluate product shelf life as outlined in DPC 10, *Maintaining and Testing Fluid Milk Shelf Life*. Regardless of the source, testing procedures should be selected to provide useful information relative to the product and its defects. Procedures that might be used for evaluating fluid milk line-samples include standard plate count, laboratory pasteurization count, coliform bacteria count, psychrotrophic count, gram-negative bacteria count, and stress tests.

#### Standard Plate Count using Standard Methods for the Examination of Dairy Products (SMEDP)

In most instances, standard plate counts (SPC) on freshly pasteurized milk products, either line samples or packaged products, are of little value except as bench marks to indicate the number of bacteria that survive pasteurization. However, deviation from "normal" SPC values for fresh product indicates that something has changed either in the raw milk supply (i.e. high thermoduric count) or in the processing system. In some cases, a measurable increase in the SPC of successive line samples isolates the source of contamination. But, in most cases, the level of contamination is too low to be determined by conventional SPC procedures without additional testing. SPC procedures are most useful when used with a stress test.

#### Thermoduric or Laboratory Pasteurization Count using SMEDP

Lab pasteurization counts (LPC) are most useful in evaluating raw milk as a source of thermoduric bacteria which may contribute to high SPCs in fresh pasteurized product. Raw milk should be evaluated with LPC on a routine basis. When troubleshooting high SPCs in fresh pasteurized milk, sampling areas that should be considered include the raw storage tanks or silos, the balance tank and the HTST unit after raw regeneration. If high LPCs are found, raw milk from storage tanks, tank trucks and, if necessary, individual producer samples should be evaluated as they are received. LPCs can also be used on pasteurized



product in comparison to the SPC to determine if the high counts are due to thermophilic organisms. It's important to understand that LPCs may differ from the corresponding fresh SPC values of HTST milk due to the differences in time and temperatures used.

### *Psychrotroph Count and Gram-Negative Bacteria Count using SMEDP*

Contamination after pasteurization with psychrotrophic bacteria are generally the cause of most shelf life problems in fluid milk. The most common psychrotrophic organisms implicated in the spoilage of fluid milk are gram-negative rods, primarily belonging to the genus *Pseudomonas*. Contamination of this sort is most often the result of inadequacies in the cleaning and sanitation program. As a rule, gram-negative bacteria do not survive pasteurization. This is why coliform bacteria, which are one type of gram-negative bacteria, are used as indicators of poor hygiene in pasteurized milk products (see Coliform Bacteria Count). The psychrotroph count (SPC procedure incubated at 7°C for 10 days) will detect gram-negative and gram-positive psychrotrophs, though gram-positive organisms rarely cause problems in pasteurized fluid milk (see Thermophilic Psychrotrophs). The gram-negative bacteria count with Crystal Violet Tetrastazolium Agar (CVTA) selects for gram-negative bacteria (see appendix). Since contamination levels are generally very low, gram-negative bacteria counts are most useful when used with a stress test.

### *Coliform Bacteria Count using SMEDP*

Coliform bacteria counts using the Violet Red Bile Agar (VRBA) procedure are used extensively in the dairy industry as indicators of post-pasteurization contamination due to poor cleaning and sanitation. Where coliform levels are detectable in the finished product, coliform counts on line-samples from the pasteurized side of processing can help determine the source of contamination. Low level coliform contamination can often be detected after milk has been pre-incubated (i.e. 37°C for 6 hrs.) prior to plating on VRBA.

### *Thermophilic Psychrotrophs*

Thermophilic psychrotrophs occasionally spoil milk in the absence of gram-negative contamination. These types of organisms generally grow much slower, causing problems later in shelf life. To detect thermophilic psychrotrophs, milk that is laboratory pasteurized, is plated for psychrotrophic organisms as well as for the standard LPC. Alternatively, the heated milk can be stored at 7°C for 10 days and then plated for SPC where a significant increase over initial LPC indicates the presence of thermophilic psychrotrophs.

### *Yeast and Mold Counts using SMEDP*

Yeast and mold, though rare, may occasionally cause problems with pasteurized milk. More often they are associated with paper contamination in ultra-pasteurized products. Detection generally requires an extended stress test such as holding milk 14 days at 7°C.

### *Stress Tests*

In most cases, psychrotrophic spoilage organisms recontaminate the product at very low levels, often less than 1 per ml, below the level of detection of most plating procedures. This still presents a major concern because one psychrotrophic bacterium with a doubling time of 6 hours can spoil a quart of refrigerated milk in less than ten days (counts of greater than 10 million per ml). The SPC procedure generally is not sensitive enough to detect low-level contamination in line-samples or finished product. The number of psychrotrophs may only be a small proportion of the total SPC and are indistinguishable from non-psychrotrophic bacteria with this procedure. Therefore, finding low-level contamination is a difficult task. It generally requires a large sample size and an incubation period or "stress



test" which selects for psychrotrophic organisms and allows them to increase to detectable levels.

Another advantage of stress tests is that they allow resuscitation of "injured" microorganisms, which can repair themselves during milk storage, allowing subsequent growth and possible spoilage. Microorganisms in milk may be in an injured state due to previous exposure to heat, drying, chemical sanitizers or other environmental stresses. Injured organisms are less able to grow in selective media such as VRBA. Repair mechanisms, however, allow impaired bacteria to grow in microbiological media that may otherwise inhibit them.

To detect low level contamination and/or injured microorganisms, a number of modified tests or stress tests, have been developed. Suggested tests as described in DPC 10, *Maintaining and Testing Fluid Milk Shelf Life*, include:

*Mosely Keeping Quality Test*

Incubate product 7 days at 7°C. Evaluate with SPC, coliform or gram- negative bacteria count.

*Virginia Tech Shelf Life Test*

Incubate product at 21°C for 18 hours. Evaluate with SPC, modified SPC (21°C for 48 hrs.) or gram-negative bacteria count.

*Preliminary Incubation VRBA Test for Coliform Bacteria*

Incubate the product at 37°C for 6 hrs. Evaluate with the Standard VRBA procedure for coliforms.

Interpretation of these and additional procedures are outlined in DPC 10, *Maintaining and Testing Fluid Milk Shelf Life*. The T.T.C. (triphenyl tetrazolium chloride) Test for the shelf life prediction of fluid milk products (a dye reduction test similar to, but more refined than, the old methylene blue and resazurin tests) is described in the appendix.

### Recommended Procedure for Fluid Milk Line-Sampling

1. Collect the finished product and line-samples as described, taking a minimum 50 ml sample at each sampling site. Include raw milk samples if thermophilic bacteria are a concern (i.e. high fresh SPCs). Sampling may be done at the beginning and end of processing to determine the influence of running time.
2. Transfer milks to sample vials or Whirl-Pak bags with sufficient head space. Plate for SPC and coliform, avoiding contamination of the sample. Include lab-pasteurization counts of raw milk and, if necessary, pasteurized milk samples if thermophilic bacteria are the problem.
3. Store the remainder of the milk at 21°C for 18 hours or 7 °C for 7 days. After the selected incubation period, plate for SPC, modified SPC (see DPC 10, *Maintaining and Testing Fluid Milk Shelf Life*), or gram-negative bacteria count.

## **Product Line-Sampling Non-Fluid Dairy Products**

### Suggested Sampling Sites



To list the product line-sampling strategies for every dairy product is beyond the scope of this guideline. The general design of a sampling program is to isolate critical areas in processing where contamination can occur so the source of contamination can be identified. This involves collection of samples at steps that are either chronologically or physically separated. For cottage cheese for example, samples might consist of pasteurized skim, whey after cooking, curd wash water, drained curd ready for creaming, pasteurized cream dressing, creamed blended product from the vat or blender and finished packaged product.

### Sample Collection

Sample collection methods and storage conditions will also differ depending on the nature of the product. The nonhomogeneous nature of many dairy products will warrant different sampling procedures than those used for fluid milk. The most recent edition of *Standard Methods for the Examination of Dairy Products (SMEDP)* should be consulted for how to obtain a proper representative sample.

### Microbiological Testing

Selection of microbiological tests for the evaluation of line-samples and finished product will vary depending on the nature of the product. High-moisture dairy products, such as cottage cheese, can be subject to spoilage by gram-negative bacteria. Cottage cheese and other fermented products with lower pH values are selective for yeast and molds, which generally are not a problem in fluid milks. In other products, such as cheddar cheese, the types of organisms that cause spoilage are selected for by lower pH, organic acids, lower moisture, higher salt, and the presence of other inhibiting factors. Dry dairy products, because of limited moisture, are generally not subject to microbial spoilage, though contamination with pathogenic organisms is of concern and should be monitored. A listing of suggested microbiological methods for evaluating different dairy products follows:

Cultured Products – coliform count, yeast and mold count, psychrotroph count, gram-negative bacteria count.

Ice Cream Mix – standard plate count, laboratory pasteurization count, coliform bacteria count, psychrotroph count, yeast and mold count, stress tests (Mosley, Preincubation Test).

Cheese – yeast and mold count, coliform count. Also test for gas producing bacteria such as heterofermentative. Lactobacilli, Leuconostoc spp., lactate-fermenting, Clostridia (in raw cheese milk).

## **Interpretation of Line-Sampling Results**

Line samples taken prior to the source of contamination should have a relatively low Standard Plate Count (SPC) and should show little increase in counts after any stress tests are performed. Also, indicator organisms, such as gram-negative bacteria, coliform, yeast, or molds should not be detected in either the fresh or incubated samples. Line-samples taken after the contamination source often show an increase in bacteria counts relative to previous samples which may be evident in the fresh sample or only after the stress test. Indicator organisms often become evident as well. The source of contamination can then be narrowed down to the area between the last sample without contamination and the first sample with the contaminant. Equipment should be inspected, and the cleaning and sanitation procedures should be re-evaluated for this area.



## Product Packaging Evaluation

Microbiological contamination of packaging material is generally not a problem as long as proper storage and handling practices are followed. However, mandrels and other components on paper-forming machines can be a source of contamination if not kept clean, sanitized and in good condition. The influence of packaging material as a source of microbial contamination can be determined by testing representative samples of preformed packages, blow-mold bottles or formed and sealed paper cartons taken off the filling machine (without product). The testing procedure involves plating a solution used to rinse the container. The procedure, as described in *SMEDP*, is outlined below.

### Rinse Solution

Rinse solutions for the evaluation of packaging materials are generally formulated to inactivate residual sanitizers, including halogen (chlorine, iodine), quaternary ammonia, and acid sanitizers. Neutralizing Buffer is recommended as a convenient dehydrated formulation. Alternatively, an effective neutralizing buffered rinse solution can be made as follows:

#### Buffered Rinse Solution

1.25 ml Stock Dilution Buffer

5.0 ml 10 % Sodium Thiosulfate

4 grams Azolectin

10 grams Tween 80

Make to 1 Liter with Microbiologically Suitable (MS) water Adjust the pH to 7.2 Autoclave at 120°C for 15 min.

Sterile Nutrient Broth can also be used and is recommended if an incubation period is used to amplify low levels of microorganisms. **If Petrifilm is used DO NOT use rinse solutions containing thiosulfate or sodium citrate** as this interferes with the formation of visible colonies. Lethen Broth is the recommended buffer when using Petrifilm counting procedures.

### Addition of Rinse Solution

For preformed, capped packages (i.e. blow-mold plastic):

1. Wipe the area around the cap with 70 % ethanol to sanitize, allowing 1-minute exposure time.
2. Remove the cap, add the appropriate amount of rinse solution (see Table 3) and recap.

For sealed paper cartons:

1. Swab an area on the paper surface with 70 % ethanol, allowing 1-minute contact time.
2. Fill a sterile syringe with the appropriate amount of rinse solution (see Table 3) and inject directly into the carton at the site sanitized. Remove the syringe and seal the hole with cellophane tape.

**Table 3. Recommended Volume of Rinse Solution**

Package Size	Volume Rinse Solution
Quart or Less	20mL
Half-Gallon	50mL
Gallon	100mL



### Rinse Procedure

1. Hold the container so that the long axis is horizontal, and shake 10 strokes of approximately 20 cm.
2. Turn the container 90° and repeat. Turn two more times and repeat so that all sides of the carton are exposed to the shaking procedure.

### Sampling Procedure

1. Wipe the area surrounding the cap or opening spout of the container with 70 % ethanol, allowing 1-minute contact time.
2. Open the package, preventing contamination, and pipette the desired amount of rinse solution for the selected microbiological methods.

### Recommended Microbiological Method

1. Neutralizing Buffer: Rinse package as described above. Plate neutralizing buffer for SPC and/or gram- negative bacteria count.
2. Nutrient Broth: Rinse package as described above. Incubate container 21°C for 18h and plate nutrient broth for SPC and/or gram–negative bacteria count.

## **Hygiene Monitoring – Evaluation of Product Contact Surfaces**

Though a product contact surface may appear clean to the eye, the surface may still be soiled with food residue and microorganisms. This can be made evident by determining the bacterial load of product contact surfaces using procedures outlined in *SMEDP* such as direct agar contact and surface swabbing procedures. These are conventional microbiological procedures that take 2 or more days for results. The effectiveness of cleaning and sanitation programs can be determined more rapidly with systems that utilize ATP-bioluminescence technology. ATP-bioluminescence systems require surface swabbing as with conventional procedures. However, results with these systems are obtained in less than five minutes. An additional advantage of ATP-bioluminescence hygiene monitoring systems is that they also detect residual food soil as well as contaminating microorganisms. The method of choice depends on the nature of the surface to be tested and the desired speed and sensitivity of the results. Cost of methods may also be a serious consideration. The methods are outlined below.

### Sampling Sites

Hygiene monitoring should be used to evaluate areas that have been shown or are suspected to be likely sources of contamination. Areas that are hand-washed or are difficult to clean, dead ends, filler valves, gaskets, plug valves, mandrels, metering pistons and tank walls are a few sites worthy of more attention.

Note: Regardless of the methods used, product contact surfaces should be rewashed and sanitized after the evaluation procedure.

### Direct Agar Contact Methods

Direct agar contact methods are generally limited to the evaluation of smooth, flat surfaces where the total surface of the agar can contact the sampling site. Areas that can be evaluated with this method include milk storage tanks, cheese vats, mandrels and other relatively smooth, flat surfaces.



Sterile agar media when pressed to a smooth surface will pick up microorganisms present at the contact site. After incubation, colonies will form on the agar where bacteria were attached. A clean surface should have little or no signs of bacteria on the surface. More than 10 CFU per 50cm<sup>2</sup> is unacceptable.

### Replicate Organism Direct Agar Contact (RODAC) Procedure

1. RODAC procedure involves a 25cm plate. Plates are poured with Standard Methods Agar or other more selective media (i.e. Yeast and Mold) so the meniscus of the agar is above the rim of the plate. Appropriate neutralizers should be incorporated in the media to inactivate residual sanitizers. Allow 30 minutes for plates to harden and dry before use. Plates not used immediately can be wrapped and stored refrigerated for future use.
2. Lightly press the solidified agar against the food contact surface with a rolling motion so the entire agar surface contacts the sampling site.
3. Replace the cover and incubate the plate according to the selected procedure. Count CFU and record as number per 25cm<sup>2</sup>.

### Petrifilm Direct-Contact Procedure

1. For agar contact procedures the Petrifilm must be rehydrated with 1 ml of an appropriate diluent such as Standard Methods phosphate dilution water, Butterfield's buffer, 0.1 % peptone water or letheen broth. Diluents containing thiosulfate or sodium citrate should not be used. Letheen broth is recommended for testing sanitized surfaces. Allow a minimum of 30 minutes for gel formation. Rehydrated plates can be stored up to 14 days in a sealed bag at 2-8°C.
2. After gelation has occurred, open the Petrifilm by slowly lifting the top film. The agar gel should adhere to the top film. Contact the test surface while firmly rubbing the film side to ensure complete contact.
3. Lift the film from the surface and rejoin the top and bottom sheets. Incubate the Petrifilm according to the method or media used. Count and record CFUs per 20 cm<sup>2</sup> for Petrifilm Aerobic, Coliform and E. Coli Count Plates or CFUs per 30 cm<sup>2</sup> for Petrifilm Yeast and Mold Count Plates.

### Commercial Direct-Contact Products

Commercial products are available for surface contact evaluation that are convenient and simple to use. Some of the products are interpreted by chart comparison while others are counted by conventional procedures.

### Surface Swab Methods

The swab technique can be used to evaluate the microbial cleanliness of irregular as well as smooth product contact surfaces. The advantage of swabbing over direct contact is that swabbing is amenable to areas that might be more difficult to clean such as valves, gaskets, small openings, or other surfaces which are less accessible. Swabs are made of cotton, dacron, or calcium alginate attached to a breakable applicator stick. Calcium alginate is soluble in 1% sodium hexametaphosphate, sodium glycerophosphate or sodium citrate which allows complete release of organisms recovered from the test surface.



### Swabbing Procedure

1. Dispense 5mL of rinse solution (i.e. neutralizing buffer) in a screw cap test tube (7-10 cm) and sterilize. If Petrifilm is used in the plating procedure, the rinse solution should not contain thiosulfate or sodium citrate (i.e. use letheen broth).
2. Remove the sterile swab from packaging, touching only the end of the stick. Moisten the swab in the buffer solution and drain the swab by pressing on the side of the tube. Hold the swab head against the test surface so the handle is at a 30° angle with the surface.

For irregular surfaces, such as gaskets and valves, swab as much of the area of interest as possible.

For smooth surface, such as tanks and vats, rub the swab firmly over an 8x2 (50 cm<sup>2</sup>) area three times and return to the rinse solution. Rinse the swab briefly in the solution with a twirling motion. Drain the swab against the tube wall and repeat for four more areas.

3. After the last area has been swabbed break the stick so the swab head remains in the rinse solution and seal the test tube for transport to the laboratory.

Note - For comparable results, it is important to consistently swab the same size area during each testing period. If not immediately plated, cool the samples to 0 to 4.4°C and test within 36 hours.

### Plating Swab Solutions

Shake the sample 50 times in 10 seconds in a 15cm arc while hitting against the palm of the hand. Plate 1mL or the appropriate dilution.

### Counting and Reporting

The number of microorganisms reported from a swabbed area is calculated based on the area(s) swabbed, the dilutions used, and the amount plated.

#### Irregular Surfaces

If a singular irregular surface is swabbed with 5mL rinse solution, the CFU per mL of rinse solution should be multiplied by 5 and reported as CFU per item or total area swabbed.

#### Smooth Surfaces

If five 8x2 (50cm<sup>2</sup>) areas or five equal irregular surfaces (i.e., five gaskets) are swabbed per tube report the CFU per mL of rinse solution plated as CFU per 8x2 (50cm<sup>2</sup>).

### Interpretation

The number of microorganisms recovered from properly cleaned and sanitized food contact surfaces should be minimal. Counts of greater than 10 per 8x2 (50 cm<sup>2</sup>) are considered unclean. Cleaning procedures should be reevaluated.

## **ATP – Bioluminescence Methodology**

The ability to rapidly detect failures in a cleaning and sanitation program has obvious advantages in a dairy operation, allowing rewashing of unclean surfaces before processing. ATP-



bioluminescence hygiene monitoring systems offer a means of obtaining a quick evaluation of product contact surfaces. Not only is ATP- bioluminescence more rapid than conventional microbiology, with results in less than 5 min., it also has the added advantage of detecting food residues as well as microbial contamination. These capabilities make ATP-bioluminescence a valuable tool in a quality program.

Testing procedures using ATP-bioluminescence are based on the detection of adenosine triphosphate (ATP), a biochemical present in all living cells, including microorganisms and cells associated with foods (i.e. milk somatic cells, meat, and plant tissue). ATP-bioluminescence systems measure ATP using the same enzyme system (luciferin/luciferase) that the firefly uses to produce light or "bioluminescence". The basic reaction is:



The amount of light generated, most often measured in Relative Light Units (RLU), is directly proportional to the amount of ATP present due to contamination of a surface with microorganisms or food residues. This light measurement is then compared to an established baseline level, with higher readings indicating a potentially unclean surface.

ATP-bioluminescence hygiene monitoring procedures require surface swabbing of a recommended area following the same techniques outlined for microbiological procedures. Product contact surfaces are sampled using standard swabbing techniques, a specified area for smooth surfaces or as much of an area as practical for irregular surfaces (i.e. valves or gaskets). The swab is then immersed in a cell lysing, ATP-releasing agent. Luciferin-luciferase are then added and allowed to react with any ATP recovered during swabbing. The resulting light is measured on an illuminometer and compared to an established cut-off value; the more ATP (microorganisms or food residues) present the higher the light reading. A swabbed surface with a light reading over the cut-off value is interpreted as being soiled and should be re-cleaned. Examples of ATP-bioluminescence readings from one system compared to conventional microbiology are shown in Table 4.

**Table 4. ATP-Bioluminescence vs. Conventional Microbiology**

<b>Area Swabbed</b>	<b>RLU<sup>2</sup></b>	<b>SPC<sup>3</sup></b>	<b>Gram-Neg<sup>4</sup></b>	<b>Coliform</b>
Pipeline from PMT	119	55	<1	<1
Pipeline to Filler	26	<1	<1	<1
Filler Bowl	36	<1	<1	<1
Filler Rubber	100	30	<1	<1
Filler Valve Stem	8,900	11,000	1,700	114

There are several ATP-bioluminescence hygiene monitoring systems available. All are supplied with the needed reagents and equipment, including an illuminometer (light meter). The ease of use, cost and versatility vary among available systems, though all kits involve the same general steps and appear to be relatively comparable in sensitivity. Most of the systems claim sensitivity to levels of bacteria greater than 1000 CFU or yeast greater than 100 CFU. This would suggest that ATP-bioluminescence procedures are not as sensitive in detecting microbial contaminants as standard microbiological methods. However, cut-off values are based on total ATP which includes the detection of non-microbial ATP or food residues that supports microbial growth. This ability

<sup>2</sup> Relative Light Units, established cut-off 100-150 RLU.

<sup>3</sup> SPC from a swab of an adjacent site, may differ in contamination load from ATP swab.

<sup>4</sup> Gram-negative bacteria count determined with CVTA.



to detect food soil along with rapid results are distinct advantages of the hygiene monitoring systems over conventional methods.

## **Environmental Sampling**

The overall evaluation of a dairy plant's sanitation program should include the analyses of environmental samples taken throughout the plant. A solid environmental sanitation program designed to minimize sources of microbial contamination is as important as the cleaning and sanitizing of the processing equipment.

### **Environmental Surface Sampling**

Potential pathogens and most spoilage organisms occur in dairy products as post-processing contamination. The potential for these types of organisms to originate from the plant environment is a strong possibility. Pathogens such as *Listeria* and *Yersinia* have generally been associated with pooled water/product wet areas such drains, floor mats, condensate, conveyor systems. Poorly maintained coolers have been found to be important potential reservoirs for psychrotrophic pathogens.

Many testing laboratories provide environmental testing kits with instructions and suggestions for use. Swabbing sanitized surfaces should be done with an appropriate neutralizing medium or buffer which will inactivate the sanitizing agent used.

### **Air Sampling**

Microbial air quality in a dairy plant can influence the quality of the finished product. This is especially true in packaging areas, in areas associated with ice cream freezer air intakes and in areas where the product is open to the environment, such as in cheese vats. Airborne contamination in dairy operations has been associated with heating, ventilation, and air conditioning systems (HVAC), personnel traffic, boxing and palletizing operations, and the creation of aerosols. Standing water and spilled product become breeding grounds for microorganisms that can become airborne. Production practices should be such that aerosols are not created during product processing times and that overall sanitation procedures are followed to minimize the microbial load in the plant environment. Microbiological air quality should be monitored on a daily basis while deviation from established standards should signal that a potential environmental hazard exists.

There are a number of options available to monitor microbial air quality. The simplest method is by sedimentation, which is generally done by opening a petri dish or Petrifilm with Standard Methods Agar or a selective medium for a specified amount of time, usually 15 minutes. Counts are recorded as CFU per area of plate per time of exposure. Sedimentation, however, is very dependent on air currents, traffic, particle density, and other factors that will affect the sedimentation rate. Generally, this is most useful when evaluating the amount of bacteria settling on a specific surface or piece of equipment.

Other methods available for testing the microbiological quality of air include impaction onto a solid medium (slit or sieve type), centrifugation impaction, filtration, impingement in a fluid, and electrostatic precipitation. These procedures work by entrapment and enumeration of particles from a measured volume of air. The number and the types of organisms recovered will vary among the different air testing procedures.

Regardless of the method of analysis, the key to monitoring microbiological quality of air within a dairy plant environment is to establish a "normal range" for microbial numbers of a



"clean plant" with the method in use. When counts exceed these established values, it suggests that there is a failure in the environmental sanitation procedures in that area.

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## APPENDIX

### T.T.C. Test

The T.T.C. test is used as a rapid test for predicting the approximate shelf life of stored pasteurized milk.

#### Reagents

- Nacconal NRSF (sodium alkylaryl sulfonate) – active agent which inhibits the growth of Gram-positive organisms without effect on Gram-negative organisms.
- T.T.C. (triphenyl tetrazolium chloride) – reduction indicator of bacterial activity.
- K<sub>2</sub>HPO<sub>4</sub> (0.5%) + KH<sub>2</sub>PO<sub>4</sub> (0.001%) – buffer to maintain the pH of the milk to prevent acid from interfering with the reduction of TTC.

#### **Preparation of TTC Solution**

TTC	0.1gm	KH <sub>2</sub> PO <sub>4</sub>	0.1gm
Nacconal	1.0gm	d-H <sub>2</sub> O	100mL
K <sub>2</sub> HPO <sub>4</sub>	5.0gm		

Dissolve all ingredients in distilled water, autoclave and store in a brown bottle.

#### Test Procedure

1. Aseptically transfer 10mL of milk sample into a sterile screw cap test tube. Close tightly and store under refrigeration.



2. After all samples have been collected, add 1 ml of TTC solution to each tube and mix well (avoiding contamination).
3. Incubate tubes at 20°C for a total of 48 hours checking at 12 hours, 18 hours and 24 hours.
4. Test is positive at the first sign of a pink color throughout. Note: pink color at the bottom of the tube with no pink color throughout is read as a negative.

### Controls

Positive - use 10 ml of cultured buttermilk instead of milk.

Negative - use 10 ml of sterile milk.

### **Interpretation of the TTC Test**

<b>Color Reduction Lifetime</b>	<b>Approx. Shelf at 4.4°C</b>
12 hours	<4 days
18 hours	6 days
24 hours	9 days
48 hours	15 days

## **Crystal Violet Tetrazolium Agar for Gram-Negative Bacteria Counts**

### Reagents

#### **Crystal Violet Solution, 0.1% in ethanol:**

Dissolve 0.1 gram crystal violet in 100mL of 95% ethanol.

#### **2,3,5, triphenyl tetrazolium chloride solution:**

Dissolve 1 gram 2,3,5, triphenyl tetrazolium chloride in distilled water and **filter sterilize** (0.2 micron). Store refrigerated.

### Crystal Violet Tetrazolium Agar

1. Prepare Standard Methods Agar according to the manufacturer's recommendations.
2. Add 1 mL of 0.1% crystal violet solution per liter of agar after boiling (1 ppm final concentration). Dispense and sterilize at 120°C for 15 min. Do not over sterilize.
3. After agar has been sterilized (or remelted) cool to 44-46°C. Add 5 ml of 1% aqueous solution of 2,3,5, triphenyl tetrazolium chloride per liter of agar (50 ppm final concentration). Mix gently.
4. Use agar in standard plating procedures. Incubate at 32°C for 48 hours or 22°C for 5 days. Count dark red colonies. Report as Gram-negative bacteria per gram or ml of sample.

**NOTE:** Certain yeast and molds as well as some gram-positive cocci will grow on the media but produce atypical colonies (light pink, colorless). When in doubt confirm colonies with a Gram-positive.



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