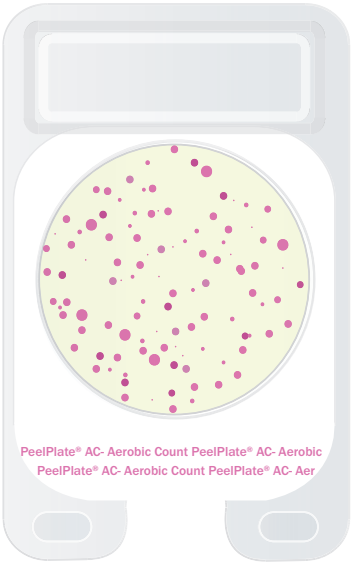
CHARM SCIENCES INC

NOTHING WORKS LIKE A CHARM



PeelPlate®

ENVIRONMENTAL SAMPLING GUIDE

GUIDE TO USING BACTERIAL INDICATORS IN ENVIRONMENTAL SAMPLING OF FOOD PLANTS

OVERVIEW

Environmental sampling in a food plant encompasses microbial testing during production operations and post-sanitation. It can incorporate surface testing in production zones as well as air and water monitoring.

This guide is a simplified explanation of how to environmentally sample for microbes in a food plant. It discusses how to sample, test and interpret indicator tests, such as aerobic, heterotrophic, coliform, Enterobacteriaceae, and yeast and mold results. This data may be used to develop, improve, and verify the effectiveness of hygiene and sanitation programs designed to reduce the risk of pathogens in food production. Additionally, data may be used to monitor production runs for microbial risks that may be used for extended run validation and shelf life improvement.

SURFACE SAMPLE AND TESTING

There are a number of different types of sampling devices and buffers for sampling surfaces. The selection of the type will vary on customer SOPs, needs and costs. Charm Sciences offers 3 varieties of sample devices:

- Sponge in bag rehydrated with 10 mL buffer. Sponge in sample bags are typically used in applications where samples are sent to outside labs for pathogen tests. Surfaces can vary from food contact surfaces to large animal carcasses during processing. The sponge is attached to a stick that is detached after sampling.

- Sample swab rehydrated with 1 mL Buffer. Swab sampling tubes may be used in applications where a surface or site is sampled and plated on a 1mL indicator test. The volume is convenient to rehydrate a 1mL indicator test, e.g. Peel Plate microbial test, without need for measuring 1 mL. The swab and buffer are in a closed screw cap container, convenient for sample collection, delivery, and subsequent testing.

- Sample swab rehydrated with 10mL Buffer. Swab sampling tubes may be used in applications where a surface or site is sampled and plated on multiple indicator tests, e.g. aerobic, Enterobacteriaceae, and yeast/mold, pathogen, or higher sensitivity tests.

- A variety of buffers may be used for environmental sampling, each containing specific ingredients for an assortment of purposes. Charm Sciences utilizes HiCap Broth, which effectively neutralize sanitizers that may be present on the sampling surface for efficient bacterial recovery, and neutralizing buffer, which contains fewer nutrients; the choice of buffer might be influenced by recovery of target and non-target organisms as well as the amount of sanitizer residual in the test matrix.

- Alternative buffers include peptone, Leethen Broth and DE Broth supplied by alternative vendors.

SPONGE IN BAG SAMPLING

1

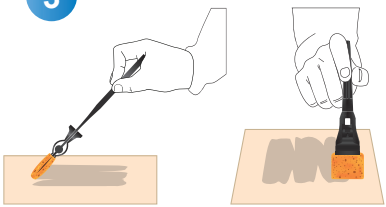
Label the sample bag.

Optional: Put on sterile gloves in order to further reduce risk of user cross-contamination.

2

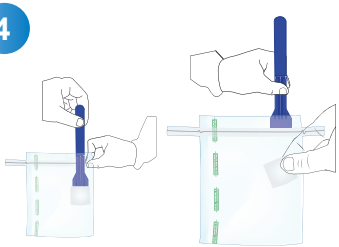
Tear open twist-seal bag to recieve sponge.

3



Grasping stick, press firmly to survey 10 cm x 10 cm (100 cm^2)surface with the sponge end.

4

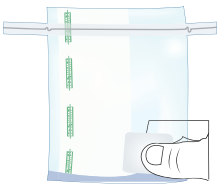


Place sponge and handle part way into opened bag.

Unclip the sponge by pressing the clips through the bag walls and pushing stick toward sponge. Remove the stick.

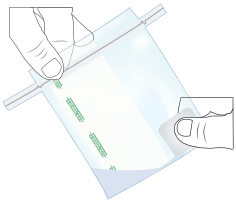
Sponge in Bag Testing Procedure for Microbial Indicators

1



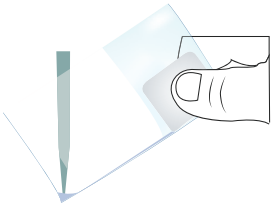
Handle sponge on the outside of the bag. Squeeze and reabsorb liquid 3 to 5 times.

2



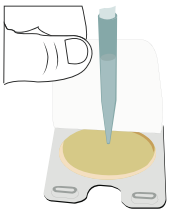
Tilt corner of bag upwards and squeeze sponge to rinse liquid to opposite corner of bag. Let any food particles settle to bottom of bag.

3



Open bag and pipet 1 mL of liquid. Avoid uptake of any food particulates as these may clog the pipet or cause interferences with the microbial test.

4

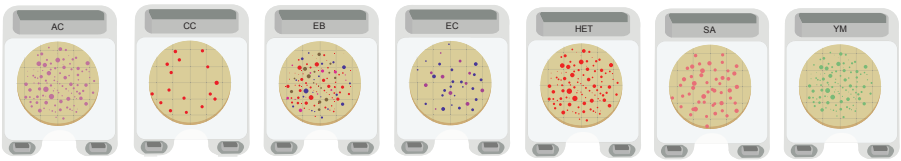


Lift cover and vertically pipet 1 mL sample in 2 to 3 seconds.

Reseal adhesive cover.

5 Incubate plates according to time and temperatures listed below:

Peel Plate Test	Incubation Times	Incubation Temperature
(AC) Aerobic	48 hours	32 or 35 °C
(CC) Coliform	24 hours	32 or 35 °C
(EB) Enterobacteriaceae	24 - 48 hrs	37 °C
(EC) Coliform/*E. coli*	24 hours	35 °C
(HET) Heterotrophs	5 - 7 days	25 °C
(SA) *S. aureus*	24 to 48 hours	37 °C
(YM) Yest and Mold	3 to 5 days	At room temperature or 25 °C in the dark



Read Results

To determine the number of bacteria present in the sample, multiply the number of colonies present on the plate by the amount of buffer in the swab bing device, then divide by the area swabbed (suggested: 100 cm^2). Use the following equation to report your final count:

$$\frac{\text{Colonies present on plate x mL buffer in sampling device}}{\text{Area swabbed } (cm^2)}$$

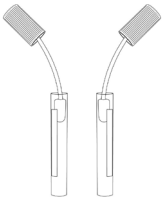
To estimate the number of colonies on a plate, count the colonies in 1 square (representative of the sample), and multiply by 17.4. Use the same equation as above to obtain an estimated count of bacteria present in sample.

SWAB IN TUBE TESTING PROCEDURE FOR INDICATORS:

1

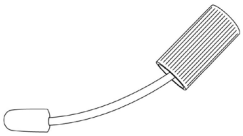
Write sample information on the tube.

2



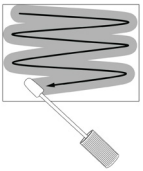
Unscrew cap from the swab tube. Press each side of the swab tip against the inside of the tube to remove excess media.

3



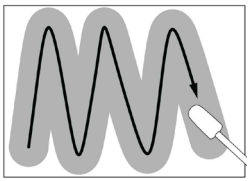
Press down firmly against surface to ensure the swab tip makes full contact with the sample surface.

4



Vigorously scrub back and forth across the surface, while rotating the swab to ensure entire swab tip makes contact with the surface.

5



Change direction 90° and repeat process.

6

Return the swab to the tube and tighten cap. Sample is ready for microbial analysis. Either send to a laboratory for analysis or use Peel Plate Microbial Tests to analyze.

***For use with Charm Peel Plate tests:**

6A: Return the swab to the tube and tighten the cap. Shake or vortex closed swab tube for 5 seconds.

6B: Unscrew cap from the swab tube. Press each side of the swab tip against the inside of the tube to remove excess media.

6C: Let any food particulates settle for 30 seconds. Pour or pipette correct sample amount onto Charm Peel Plate test.

PASSIVE AIR SAMPLING:

1. Remove adhesive cover and stick it to back foot of the Peel Plate test. Rehydrate Peel Plate test with 1.5 mL of sterile water.

2. Leave rehydrated test on a table top exposed to the air.

3. Expose the open test for 15 minutes to environmental air. Replace the adhesive cover over the plate and incubate for the specified times and temperatures.

ACTIVE AIR SAMPLING DEVICES:

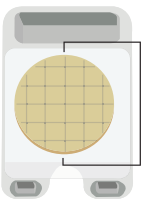
Some food plants and pharmaceutical companies utilize active air sampling pumps that circulate a certain volume of air over an open microbial test plate. This method of sampling may be chosen for increased sensitivity and controlled air volume compared to passive air sampling.

WATER AND FILTER TESTING:

Liquid beverages and water quality in food production facilities is another environmental sampling consideration. The specifications for beverages and high water quality require a 20 or 100 mL sample concentration using a microbiologic filter, e.g.45mm 0.45 µm filter. The sample is filtered in a filter housing or apparatus and then the filter is applied to growth media for microbiologic testing.

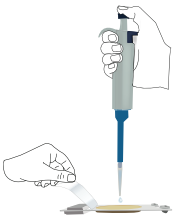
Procedure

1



- Label clear side of plate. Do not label on the 47 mm circular test area.

2



- Pull up cover to expose disk. Rehydrate with 1.5 mL of sterile water or prefered sterile broth in 2 to 3 seconds. Replace cover.

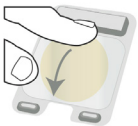
3

- Aseptically recover filter from filter apparatus using sterile tweezers.

4

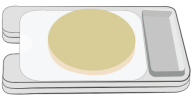
- Lift rehydrated plate cover and roll filter, grid side up, onto prepared disc.

5



- Reapply adhesive cover.

6



- Stack and incubate at appropriate incubation time.

INTERPRETATION:

Different food plants will determine their own performance specifications for indicators collected by the various sampling techniques. These specifications are based on established baselines done during production and or after cleaning. Indicators may not be correlated to pathogens, but indicators are useful in monitoring log reduction steps taken during cleaning and sanitation. They are useful in monitoring hygienic conditions during plant operation and identifying issues or events during production runs. The following are example guidelines that may be used in training programs.

PeelPlate® AC AEROBIC COUNT -Aerobic Counts

Aerobic counts are bacteria that grow in the presence of oxygen in air. In general 10 or fewer detected from a sponge sample would indicate control in sanitation, while more than 10 would indicate improvements are possible. The presence of aerobes are considered a potential indicator for risk areas for Listeria.

1

Excellent—Zero bacteria recovered

2

Very Good—One bacteria per plate (10 per 100 cm^2)

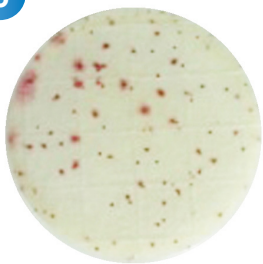
3

Marginal—Ten bacteria per plate (100 per 100 cm^2)

4

Needs improvement—40 per plate (400 per 100 cm^2)

5



Dirty—100 per plate (1000 per 100 cm^2)

6

Very Dirty—TNTC or > 250 per plate (>2500 per 100 cm^2)

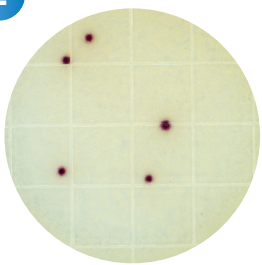
Peel Plate® CC COLIFORM COUNT - Coliform

Coliform are bacteria that are more associated with intestinal sources of contamination, fecal contamination, and may include generic *E.coli*. They are closely related to Enterobacteriaceae that include the salmonella group. These bacteria are easily killed in heat processes and should have a low incidence in foods and on surfaces. Generic *E. coli* are considered fecal source contamination indicating risk of other fecal associated pathogens like salmonella.

1

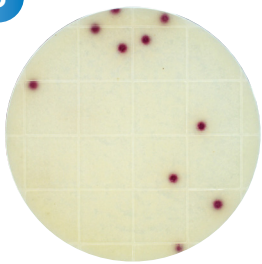
Excellent—No bacteria detected

2



Needs Improvement—Five bacteria per plate (50 per 100 cm^2)

3



Dirty—Ten bacteria per plate (100 per 100 cm^2)

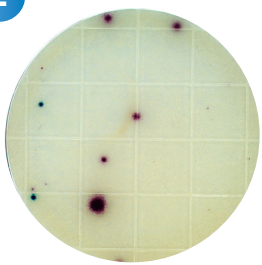
PeelPlate® EB ENTEROBACTERIACEAE - Enterobacteriaceae

Enterobacteriaceae bacteria are a family of gram negative bacteria encompassing coliform, *E. coli*, Shigella, Salmonella and Yersinia. These bacteria indicate unsanitary conditions in raw products and are killed by heat steps in food processing. EB testing is used as a post-production verification of process and pathogen risk used for example to test dry milk powder and infant formula.

1

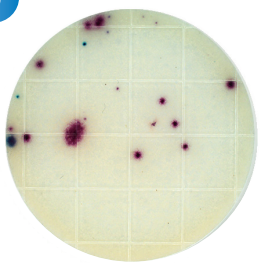
Excellent—Zero bacteria recovered

2



Marginal—Ten bacteria per plate (100 per 100 cm^2)

3



Needs improvement—Twenty-four bacteria per plate (240 per 100 cm^2)

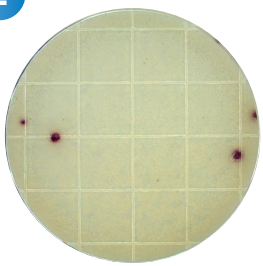
PeelPlate® EC - *E.coli* and Coliform

Coliform are bacteria that are more associated with intestinal sources of contamination, fecal contamination, and may include generic *E.coli*. They are closely related to Enterobacteriaceae that include the salmonella group. These bacteria are easily killed in heat processes and should have a low incidence in foods and on surfaces. Generic *E. coli* are considered fecal

1

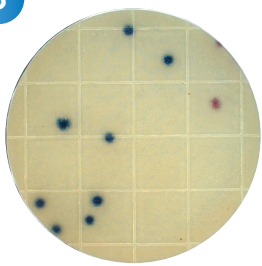
Excellent—Zero bacteria recovered

2



Needs Improvement—Five red coliform (50 per 100 cm^2)

3

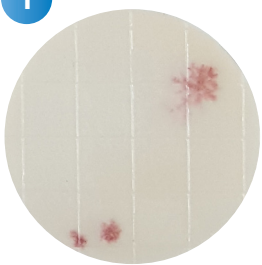


Dirty—Ten bacteria per plate. 8 blue *E. coli* and 2 red coliform (100 per 100 cm^2)

PeelPlate® HET - Heterotrophic

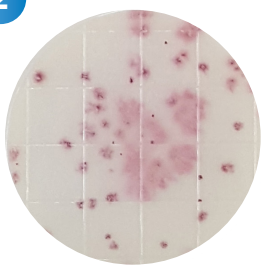
Heterotrophic bacteria are aerobes, generally mesophilic, that survive in low nutrient environments such as water. Their presence is considered and indicator of biofilm formation in piping/distribution systems. Specifications for heterotrophs in water are industry based and are as low as 5 CFU/100mL in pharmaceutical applications and as high as 5 CFU/mL for municipal water supplies.

1



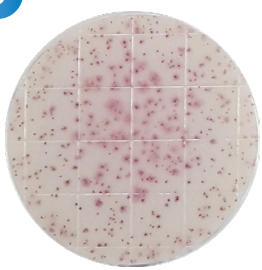
Very Good—3 bacteria per plate (30 per cm^2)

2



Needs Improvement—50 bacteria per plate (500 per cm^2)

3



Very Dirty—TNTC or >300 per plate (>3000 per cm^2)

Peel Plate® SA - *Staphylococcus Aureus*

Staphylococcus aureus bacteria are considered pathogens because they produce exotoxins in foods. They are associated with skin contact and primarily found in foods that involve human packaging such wrapped sandwiches, premade salads, soups, cut cheeses, etc.

1

Excellent—No growth.

2

Dirty—20 *Staph. a* recovered in beef stew stored for a week refrigerated.

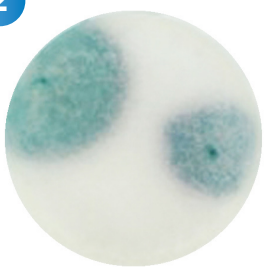
PeelPlate® YM - Yeast and Mold

Yeast and mold are microorganisms associated with food spoilage and their growth and survival are supported in damp environments. They are known to survive and spread in cooling and air systems.

1

Excellent—Zero Yeast and Mold recovered

2



Marginal—Two Yeast or Mold per plate (20 per 100 cm^2)

3

Needs improvement—more than ten per plate shown are 19 per plate (190 per 100 cm^2)

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