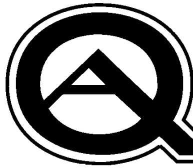
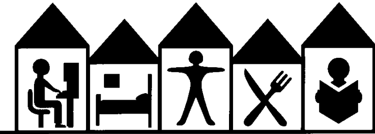




HOSPITALITY INSTITUTE OF TECHNOLOGY AND MANAGEMENT
670 Transfer Road, Suite 21A • Saint Paul, Minnesota 55114 • USA • TEL: (651) 646-7077 • FAX: (651) 646-5984
e-mail: osnyder@hi-tm.com • web site: http://www.hi-tm.com

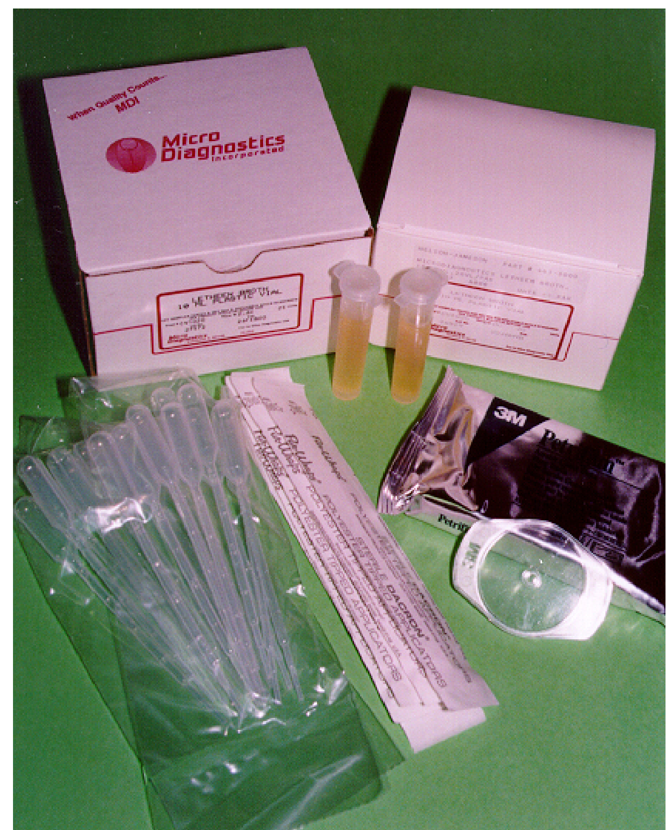
Microbiological Sampling
Food Contact Surfaces and Food

Microorganisms are one of the primary causes of spoilage and off-flavors in food products. Consequently, the production of consistent, high-quality food products requires the implementation of a thorough, well-planned cleaning and sanitizing program aimed at controlling and/or reducing the amount of bacteria entering products during and after processing / preparation. The microbial sampling and enumeration of food contact surfaces and food products can be coupled with an auditing system, which can be used for HACCP (Hazard Analysis and Critical Control Points) to evaluate and record the microbial condition of food products and food contact surfaces. In order to know that food is being processed safely, the best indicator is the Aerobic Plate Count (APC) of the food. Every menu item will have a slightly different count. However, when the APC begins to increase over a period of a week or two, the reason must be found. In the early stages of loss of control, the problem is normally a little spoilage, and foodborne illness is not an issue. By the time one can smell the problems, the process is out of control. Microbiological sampling of surfaces and food can avoid this. When a food process gets out of control, the first indicator of a problem is often an increase in spoilage bacterial count.

SAMPLING KIT

The microbiological sampling kit provides sampling tools that can be used for a convenient, reliable method to assess / enumerate bacterial contamination on retail food operations surfaces and in food samples. The kit has five components.

1. **Pouch of Petrifilm™ AC plate:** The pouch contains 50 Petrifilm™ AC (Aerobic Count) plates. These clear, polypropylene film plates contain a coating of Standard Methods nutrients, a cold-water, soluble gelling agent (guar), and an indicator dye that allows bacterial colonies to be counted. The bottom opaque sheet consists of polyethylene-coated paper, with a printed grid. When the bottom coating is wetted with 1 ml of a solution containing bacteria, and the top film is pressed in place, the guar solubilizes to form a gel on which bacterial colonies grow.
   As bacterial colonies develop on the surface of the nutrient gel, they become red due to the presence of the red indicator dye (tetrazolium). Each red dot is a colony forming unit (CFU) that is assumed to have started with one bacteria. Petrifilm™ AC plates can be used to enumerate total aerobic bacterial populations on food and food contact surfaces.
2. **Letheen broth tubes:** Each tube contains 10 ml of sterile letheen broth. Letheen broth is used for wetting swabs, because it is an accepted neutralizing agent for sanitizer chemicals that might still be present on a surface being sampled. *Note:* letheen broth is a nutrient media, and bacteria will begin to multiply in this media within a couple of hours. Therefore, when a test is started, samples should not be left in the broth for more than 1 hour before plating.



3. **Cotton swab:** When wetted by the letheen broth solution, a swab is used to wipe across or "swab" a surface and pick up a representative count of bacteria.

4. **Pipettes:** These sterile, disposable pipettes are used to transfer 1 ml of liquid from the letheen tube to the Petrifilm™ AC plate.
5. **Spreader plate:** The plastic spreader plate is used to gently apply pressure to the Petrifilm™ AC plate after the 1 ml. of sample or letheen broth has been placed on the plate. Use of the spreader plate distributes the sample or letheen broth evenly, in a circle of 20 square centimeters.

Storage and Disposal

Petrifilm™ AC plates must be handled correctly. Note expiration dates. Store sealed Petrifilm™ AC plate foil pouches at refrigerator temperatures [36 to 45ºF (3 to 8ºC)].

Each foil pouch contains 50 Petrifilm™ AC plates. After the foil pouch has been opened, store remaining, unused Petrifilm™ AC plates in a cool, dry place with the cut end of the foil pouch folded over and taped or clamped shut to prevent absorption of moisture. An opened pouch of Petrifilm™ AC plate can be stored in a freezer, because it has low humidity. Do not store an opened pouch in a refrigerator, because the dry media will deteriorate due to the humidity. For assured reproducibility, use plates within one month after the pouch is opened.

If plates are being used for classroom demonstrations and accuracy is not essential, they can be used up to 6 months after opening. Exposure of plates to temperatures above 75ºF (24ºC) and/or relative humidity above 50% can affect the overall performance of the plates. *Do not use plates that show brown discoloration*.

Store the 10-ml letheen broth tubes at refrigerator temperatures. The swabs and pipettes can be stored at room temperature.

After use, the swabs and tubes will have no more contamination than the surface that was sampled. They can be discarded in regular waste containers. The Petrifilm™ AC plates can have bacterial counts equivalent to a few grams of spoiled food. After enumeration, the film plates should be wrapped in paper or plastic film and placed in a garbage can.

SURFACE SAMPLING

Microbiological surface sampling procedures permit operators to discover both the magnitude and source of contamination entering the food preparation / food production environment. Microbiological monitoring may be carried out for one or more of the following:

1. Determination of the efficiency of sanitation procedures and cycles
2. Determination of the frequency required for sanitation cycles
3. Discovery of environmental sources of spoilage organisms that can be reduced to extend food refrigerated shelf life
4. Determination of the frequency required for special maintenance procedures (e.g., changing of air filters to reduce airborne contamination)
5. Sanitary design and condition of food equipment.[2]

DIRECTIONS FOR SURFACE SAMPLING

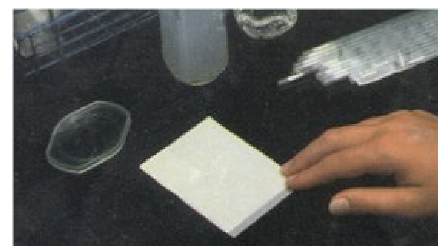
A. Petrifilm™ AC Aerobic Count Plate-Swab Method

The procedure described in the following paragraph is patterned after the methodology recommended by the U.S. Public Health Service (USPHS). Other swabbing techniques may be substituted, but there should be scientifically sound validation of any procedure.[1,2,3] Since the objective in swabbing is to find bacteria, samples should be taken where there is high probability of finding bacteria. Counting a plate with zero colonies provides little information.

Note that a careful technique such as the one described must be used during the entire recovery process to ensure accuracy.

Recommendations

1. Wash hands thoroughly with soap and warm water before and after sample collection and plating. You do not want your hands to cause the test to be invalid.
2. Wash, rinse, and sanitize surfaces on which plates are laid before and after plating. Suitable sanitizing solutions are 50 ppm chlorine (1 teaspoon of bleach per gallon of water or 1 ml of bleach per liter of water).
3. Avoid touching the swab head, stem and tip, or plate with your fingers, since your fingers will have transferable skin bacteria.
4. Avoid contaminating the swab head or open pouch or leaving it exposed for more than 5 minutes.
5. Cut open the end of the Petrifilm™ pouch. Leave the pouch intact as much as possible so that it can be folded over and clipped shut, thus saving unused film plates. Remove only enough Petrifilm™ plates from the protective pouch to do the test.



Get the appropriate number of swabs from the kit.

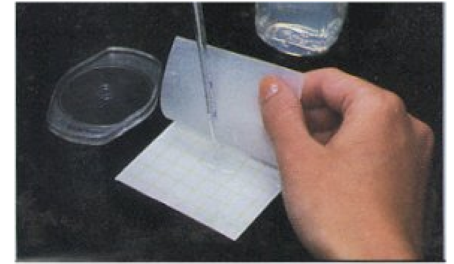
If necessary, carefully cut open the pipette bag at the bulb area of the pipette so that the bag can be folded over and clipped shut. This keeps the unused Petrifilm™ AC plates as sterile as possible.

6. Before starting to collect samples, lay out or design a sample collection plan. Mark (label) all Petrifilm™ AC plates on the lower edge of the film with an indelible marker so that you can identify each plate when you count it. Mark (label) the letheen broth tubes that you intend to use, if you do more than one at a time, to avoid mixing samples (e.g., sampling the salad prep cutting board and the slicing machine). For example, label a Petrifilm™, "8-square-inch sample / salad cutting board surface 10^{-1}." Then, label the letheen broth tube, "salad 10^{-1}." In a like manner, label the second Petrifilm™, "8-square-inch sample / slicer platform 10^{-1}," and the tube, "slicer 10^{-1}."

STEP ONE: SURFACE SAMPLING PROCEDURE

1. Remove a cotton swab from its sterile packaging. Feel the swab package and find the end without the cotton wrap. Tear this handle end open so that you can reach the swab stick handle. Holding the swab in one hand, take a letheen tube in the other hand and flip open the top.
2. Moisten the cotton swab head in the letheen broth and gently press out excess broth on the swab against the wall of the tube with a rotating motion.
3. Hold the swab at a 30º angle to the surface to be tested.
4. Rub the swab slowly and thoroughly over approximately 8 square inches (50 square centimeters). There are no standards for where to swab. The object of swabbing is to find bacteria if they are there. A good swabbing method for a surface, such as a sanitized cutting board, is to swab in two places 4 square inches (25 square cm) each near the middle of the board where food is cut. Swab the first spot using horizontal strokes. Rinse out the swab in the letheen broth by rotating and moving it up and down 5 times. Swab again using vertical strokes. Rinse the swab in the letheen broth again, using the same motion. Do the second 4 square inches (25 square cm) spot the same way for a total of 8 square inches (50 square cm).

   The United States Public Health Service (USPHS) recommendation for tableware is to swab the food contact surface of an item such as knife blade, fork tine, or spoon bowl. In like manner, the rim of the glass is swabbed first on the inside and then on the outside. In these cases, the results are stated as bacteria per fork, glass rim, etc. The area of what is swabbed is not as important as simply being accurate as to what was sampled. This way, the procedure can be repeated later to validate results.
5. After the swab is rinsed for the final time in the broth, press it against the inside of the tube to remove excess moisture. Remove the swab and throw it away. Close the tube. Mix the tube gently by inverting it 10 times. *Do not shake it, because letheen broth will foam a little.*
6. Take a transfer pipette from its sterile bag by holding the bulb and removing it from the bulb end of the package. Flip open the letheen broth tube containing the swabbed surface sample and take up 1 ml of liquid from the tube by squeezing the bulb slightly and pulling up enough liquid to reach the 1-ml line. Do not allow air bubbles in the stem of the pipette.
7. Holding the pipette with 1 ml in one hand, lift the clear top film of the marked Petrifilm™ AC plate.

   Squeeze the liquid to the middle of the Petrifilm™. Avoid air bubbles. Roll the top film down onto the sample by letting the back hinged part of the film first contact the liquid on the plate.

8. Take the plastic spreader plate in your fingers. Using the recessed side of the plastic spreader, apply a gentle, even, downward pressure on the top film above the 1-ml solution to evenly distribute sample. *Do not slide the spreader across the film.* Remove the spreader. *Do not disturb the plate for 1 minute to permit solidification of the gel.*

9. Discard the pipette and letheen broth tube containing the tested surface sample. If finished, close the end of the Petrifilm™ foil pouch, clip it shut to keep the remaining plates dry and sterile, and return pouch to refrigerator or freezer storage.

STEP TWO: INCUBATION OF SAMPLE

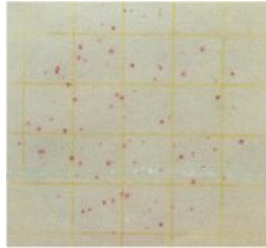
Incubate the Petrifilm™ plates in stacks not more than 20 units high. Keep temperatures controlled to +2ºF (+1ºC). *The incubation temperature range that will give the best overall indication of contamination is 68 to 77ºF, ± 2ºF (20 to 25ºC, ± 1ºC) for 2 to 3 days.* Both psychrotrophic (cold-loving) and mesophilic (mid-temperature-loving) bacteria multiply in this temperature range.

If the purpose is only to find out about the microorganisms that will spoil the food in refrigerated storage, incubate Petrifilm™ plates at a temperature range of 41 to 50ºF (5 to 10ºC) for 7 to 10 days.

If the purpose of the test is to find out about the possible presence of pathogenic (mesophilic) organisms, incubate Petrifilm™ plates at 95ºF (35ºC) for 24 to 48 hours.

STEP THREE: COLONY ENUMERATION

1. The circular growth area of each Petrifilm™ plate contains approximately 20 square centimeters.
2. Petrifilm™ AC plates should be counted in a well-lit area. A magnifying glass can be used if desired. As colonies of bacteria develop and grow, the tetrazolium indicator dye in the media becomes red. A colony represents the growth of 1 microorganism to the countable size of about 1,000,000 microorganisms. Some organisms do not utilize the dye as well as others. This results in different intensities of red. If the 8 square inches (50 square cm) meets USPHS standards, there will be less than 10 colonies on the plate. (The USPHS sanitation standard is fewer than 100 microorganisms per 8 square inches. With a 1:10 dilution, this relates to 10 microorganisms per 1 ml of liquid in the 10-ml tube.)

   To be statistically accurate, count plates with 20 to 200 colonies. On plates with counts approaching 300 colonies, estimates can be made by counting 4 1-cm squares that look representative of the film counts, and multiplying by 5 to equal the area of one plate. When plates exceed 300 counts and are essentially all pink-red, record the results as too numerous to count (TNTC). Occasionally, on overcrowded plates, the center will lack visible colonies but many small colonies will be seen on the edges. When this occurs, count a representative number of squares at the edge and multiply by the appropriate number to obtain an estimated plate count. Sometimes organisms will cause liquefaction of the gel, resulting in a "spreader." If the spreader interferes with counting, an estimate should be made from an area not affected.
3. To isolate colonies for further identification, lift the top film and pick the colony from the gel.
4. If you want to permanently record the results of a plate, you can lay a plate face down on a photocopy machine, and the red dots copy well.

B. Petrifilm™ AC Plate -- Direct Contact Method

When hydrated with 1 ml of sterile letheen broth solution, Petrifilm™ AC plates can also be used as direct contact plates for the microbiological examination of surfaces. This method only works for flat surfaces such as cutting boards and table tops.

STEP ONE: PREHYDRATION OF PETRIFILM™

(*Note:* Good aseptic technique must be employed throughout the rehydration procedure to avoid contaminating the Petrifilm™ plates.)

1. Place the Petrifilm™ plates face up on a sanitized flat surface. Label each film with the name of the flat surface that you will sample.
2. Take a tube of letheen broth and flip open the top. Take 1 ml using the pipette. [One (1) tube of letheen broth provides enough broth to hydrate 9 Petrifilm™ plates.]
3. Lift top film of the Petrifilm™ plate and transfer 1 ml of sterile letheen broth solution onto the center of the bottom film.
4. Roll the top film down over the solution.
5. Using the recessed side of the plastic spreader, apply a gentle even, downward pressure on the top film to evenly distribute the letheen broth solution. *Do not slide the spreader across the film.* Remove the spreader. *Leave the plate undisturbed for 1 minute to permit solidification of the gel.*
6. For best results, the films should be used within about 24 hours.

STEP TWO: STORAGE OF PREHYDRATED PETRIFILM™ AC PLATES

If prehydrated Petrifilm™ plates are not to be used within 24 hours, wrap the prehydrated Petrifilm™ AC plates in plastic or aluminum foilto prevent drying. Refrigerate [(36 to 46ºF (2 to 8ºC)]. Plates prepared and stored as described may be used for up to 30 days after hydration.

STEP THREE: SURFACE SAMPLING PROCEDURE

1. Lift top film of the prehydrated Petrifilm™ AC plate. Avoid touching the media area with fingers.
   Note: Occasionally, the gel may split, adhering to both the top and bottom films of the Petrifilm™ plate, when the top film is lifted. This splitting of the gel will not affect the performance of the product, and the product should be used as described below.
2. *Allow only the gel on the top film to contact the surface being tested.*
3. Place the top film in contact with the surface to be sampled. *Do not slide the film on the surface; this causes streaking of the colonies*. Gently run fingers over the entire film side of the gelled area to ensure good contact with surface.
4. Lift film from surface and rejoin the top and bottom films of the Petrifilm™ plate.
5. Incubate and count plates as described previously. In this case, there will be 20 square cm (3.1 square inches) of contact surface. Therefore, a surface that meets USPHS standards will have less than 40 colonies.

ENUMERATION OF AEROBIC BACTERIA IN FOOD

A. **Food categories and dilution:** Decide how many dilutions must be done (see the table below). Note that for a statistically valid count, the Petrifilm™ plate, after incubation, should have 20 to 200 colonies. A Petrifilm™ plate with one colony can be counted; however, the variability of the count is probably high. For each dilution, 3 Petrifilm™ plates should be cultured and enumerated for statistical validity, and an average enumeration should be calculated. Some microbiologists only do one plate to save money. The number of Petrifilm™ plates that are cultured for enumeration depends on the skill and consistency of the microbiology technique and how the data will be used. A dilution of one level above and one level below should also be performed for each selected dilution level, so that a countable Petrifilm™ plate will be achieved.

Food Category	CFU / Gram	Level of Dilution
Very clean cooked food	200-2,000	10^{-1} (10 to 1)
Very clean raw food	2,000-20,000	10^{-2} (100 to 1)
Average raw food and poor cooked food	20,000-200,000	10^{-3} (1000 to 1)
Poor quality raw food and extremely poor cooked food	200,000-2,000,000	10^{-4} (10,000 to 1)
Spoiled raw and cooked food	2,000,000-20,000,000	10^{-5} (100,000 to 1)

B. **Suplies and equipment**

1. 10-ml letheen broth tubes (1 tube for each dilution).
2. 1 to 3 Petrifilm™ plates per dilution. For example, if you are going to plate a food sample with a 10^{-4} dilution, you should also plate the food sample with 10^{-3} and 10^{-5} dilutions to be sure that there are enough colonies to count after incubation. (Thus, the total number Petrifilm™ plates required would be 3 or 9 plates, depending on the number of replications.)
3. 1 one-pint plastic zipper freezer bag. (Plastic freezer bags are more durable than regular plastic zipper bags and are less likely to break when a food sample is squashed and mixed with the letheen broth.)
4. 1 two-inch wallpaper roller. (Used to squash the food sample in the bag.)
5. 1 gram scale with a 0.1 gram range. (Used to weigh 1-gram sample in the bag.)
6. 1 pipette per sample: [Used to remove 1.1-ml samples from the bag or tube (for higher dilutions), and for placing 1 ml of samples on Petrifilm™ plates.]

C. **Preparation of food sample:** Label a plastic zipper freezer bag with the name of the sample and put it on the scale. Weigh 1.1 grams of food sample into the bag. Open a 10-ml tube of letheen broth and pour the contents of the tube into the plastic bag. Seal the bag tightly, removing as much air as possible. Squash (mash) and mix the food with the letheen broth in the bag, using the wallpaper roller, until the food is broken up in the letheen broth (about 10 seconds).

To count (enumerate) this sample (a 10^{-1} dilution), transfer 1 ml of the sample, using a sterile pipette, onto the bottom of a Petrifilm™ plate. Roll down the top covering film of the Petrifilm™ and press the top of the Petrifilm™ with the plastic disk spreader (smooth side up, grooved side down) to make a 20-square-cm "petri (media) dish". Be sure that the sample name and dilution level are written on the bottom edge of the film.

D. **Dilutions:** To do samples requiring higher dilutions, use a sterile pipette to take 1.1 ml from the 10^{-1} dilution food sample in the plastic zipper bag (described above) and transfer it to a 10-ml dilution tub of letheen broth. Thus, a 10^{-2} dilution is created. Do not shake the tube, because this will cause the letheen broth to foam. Just invert the tube 10 times. Using a sterile pipette, remove a 1-ml sample and plate it on a properly labeled Petrifilm™ plate (as previously described). For another higher dilution, take 1.1 ml of this diluted sample and and transfer to another 10-ml letheen tube. Thus, a 10^{-3} sample dilution is created. Plate on properly labeled Petrifilm™. (This procedure is used until the desired dilution is achieved.)

E. **Incubation:** Incubate Petrifilm™ plates at room temperature [70ºF (21ºC)] for 2 to 3 days to allow growth and enumeration of aerobic colony forming units (CFU) that include both spoilage and pathogenic bacteria. To promote growth and enumeration of aerobic colony forming units of mainly pathogenic bacteria, incubate Petrifilm™ plates at 95ºF (35ºC) for about 24 hours.

F. **Enumeration:** To be statistically accurate, count plates with 20 to 200 colonies. On plates with counts approaching 300 colonies, estimates can be made by counting four 1-cm squares that look representative of the film counts and multiplying by 5 to equal the area of one plate. When plates exceed 300 counts and are essentially all pink-red, record the results as too numerous to count (TNTC). Occasionally, on overcrowded plates, the center will lack visible colonies, but many small colonies will be seen on the edges. When this occurs, count a representative number of squares at the edge and multiply by the appropriate number to obtain an estimated plate count. Sometimes, organisms will cause liquefaction of the gel, resulting in a "spreader." If the spreader interferes with counting, an estimate should be made from an area not affected.

To isolate colonies for further identification, lift the top film and pick the colony from the gel.

To permanently record the results of a plate, lay the Petrifilm™ plate, face down on a photocopy machine. When copied in this manner, the red dots are seen as black dots on the grid.

HINTS FOR OTHER APPLICATIONS

The rehydrated films can be used for other bacterial contamination demonstrations. For example, take a film, lift the top, and place a hair on it. Outgrowth from the hair sample indicates the presence of bacteria on hair, particularly if hair has not been washed recently.

You can also touch a dish cloth to the gel, and students can touch the film with unwashed and washed fingers.
Note: Very often, washed fingers will show a higher count than unwashed fingers, because the skin bacteria, which are in high numbers, have been loosened. Touching food with the film will work, but only if the food has a very low bacterial count (e.g., 1,000 APC per gram). Otherwise, the prehydrated Petrifilm™ will be overloaded with colony-forming units.

Liquid food can also be sampled, but, again, counts must be low (e.g., below 200 colonies per ml). Hence, very fresh milk is fine, as is water. Liquid samples can also be diluted. For example, 1 ml of sink wash water can be diluted by putting it into a 10-ml letheen broth tube. Thus, a 1-to-10 dilution is created. If you rinse the pipette 3 or 4 times in the broth, then close and invert the tube 10 times, you can use the same rinsed pipette to take the 1-ml sample for the Petrifilm™ plate count. (*Note:* While there might be slight carryover, this procedure can be used for a training demonstration to save supplies and money.)

If anyone has any other useful ideas for using this kit, send them to the Hospitality Institute of Technology and Management, so that they can be considered for inclusion in future brochures.

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1. McGoldrick, K.F., Fox, T.L., and McAllister, J.S. 1986. Evaluation of a dry medium for detecting contamination on surfaces. J. Food Technol. 40: 77-80.
2. Richardson, G.H. 1985. Standard Methods for the Examination of Dairy Products. 15th ed. Am. Public Health Assn. Washington, DC.
3. Speck, M.L. 1984. Compendium of Methods for the Microbiological Examination of Foods. 2nd ed. Am. Public Health Assn. Washington, DC.

Microbiological Sampling Kit for 50 samples: $175.00

list quantity: ________

Please send and bill to (include phone #):	**Bill to address if different:**
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FOR MORE INFORMATION CONTACT:

Hospitality Institute of Technology and Management
670 Transfer Road, Suite 21A; St. Paul, MN 55114
TEL 651 646 7077 FAX 651 646 5984
web site: http://www.hi-tm.com e-mail: info@hi-tm.com