

Introducing the Basic Essentials For Microbiology Testing

With Hazard Analysis and Critical Control Point (HACCP) programs becoming standard in all meat and poultry processing plants, many plants which have not performed microbial testing in the past may now be required to begin sampling for *E. coli*. The transition to microbial testing will be easier than expected with 3M™ Petrifilm™ plates and a new lab equipment start-up package that includes everything needed to begin in-house testing with Petrifilm plates.

To simplify the process for customers in Canada and the United States, 3M offers basic lab equipment for environment testing, food testing, and environment plus food testing, along with a training video and literature. With a minimum investment in laboratory equipment, plants can begin their own testing immediately with existing personnel—even if they do not have a microbiology background. Petrifilm plates provide dramatic labor savings over other testing methods in food processing facilities.

Petrifilm plate testing can be used for product testing, environmental

testing—including plant floors, drains, equipment and air—verification of critical control points and validation of HACCP program requirements. Sample-ready 3M Petrifilm *E. coli* Count plates have gained national recognition as the easiest, most inexpensive official testing method available. It is recognized by the USDA Agriculture Marketing Service for official analysis.

Even plants that have never done in-house testing can begin with help from a step-by-step video which clearly explains setup and use of the laboratory start-up equipment. The package includes information on the use and interpretation of Petrifilm plate results. Additional literature deals with preparing samples, making dilutions, performing environmental surface and air tests and other helpful tips. Technical service is also available through a toll-free phone call.

For more details on how to establish on-site micro testing in the U.S., call **800-860-0022**.

Simplifying the Mega Reg: *E. coli* Testing Using 3M™ Petrifilm™ Plates



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Products

Aptly nicknamed the “Mega Reg,” the 150-plus page USDA Pathogen Reduction/Hazard

Analysis and Critical Control Point Regulation can seem intimidating. But the philosophy behind the document is straight-forward—to strengthen procedures that help prevent lapses in food quality, sanitation and safety.

Simply put, the USDA is requiring food processors to develop, implement and maintain written Hazard Analysis and Critical Control Point (HACCP) plans and sanitation Standard Operating Procedures (SOP). Many large operations have had such plans in place for years and may need to make only minor modifications to come into compliance.

On the other hand, the mandate to include microbial testing as part of the sanitation SOPs means more significant adjustments for some meat and poultry operations. Under the new regulations, *E. coli* has been selected as an indicator organism for measuring processing sanitation against a national baseline level. In addition to *E. coli* criteria, processors will be required to maintain *Salmonella* standards. *Salmonella* testing will be conducted by a USDA sampling program.



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3M™ Petrifilm™ Yeast and Mold Count Plate Receives AOAC Official Methods of Analysis Approval

3M™ Petrifilm™ Yeast and Mold Count plates have been approved by AOAC International as an Official Method (AOAC® Official Method™ 997.02, Yeast and Mold Counts in Foods—Dry Rehydratable Film Method), giving food processors a simple, efficient and approved microbial testing method for detecting food-borne yeast and mold.

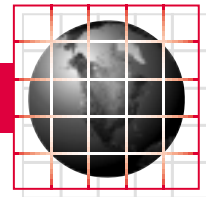
The AOAC Official Method approval recognizes the efficacy of Petrifilm

Yeast and Mold Count plates as compared to standard pour plates. Approval is based on the results of a collaborative, comparative study of six food products by 18 laboratories.

Like all 3M Petrifilm plates, sample-ready Petrifilm Yeast and Mold Count plates provide accurate counts in just three steps—inoculate, incubate and read—a labor-saving alternative to conventional testing methods. A built-in grid and an indicator dye

make counting colonies easy and consistent. Labor savings gained by using Petrifilm plates give processors more time to focus on other crucial tasks, such as monitoring critical control points or implementing HACCP programs.

News From Around The World



G'day Mate, Pass the Kangaroo Please!

An Australian meat processor is staying a jump ahead of the competition by using 3M™ Petrifilm™ plates to help ensure the quality of its exported kangaroo meat.

Southern Game Meats, Sydney, Australia, exports wild boar and kangaroo meat to markets in Europe, Asia and North America. The company began using Petrifilm plates for hygiene testing in 1993.

“We are seeing increased emphasis on the importance of hygiene in the way food products are handled, particularly for export,” said Michael Mulligan, General Manager of Southern Game Meats. “Because our products are exported to Europe and North America, we are required to satisfy the highest standards in the world.”

Productivity gains and cost savings have made Petrifilm plates an essential resource for the company. Rather than engaging an outside laboratory to perform the testing, in-house testing was selected to provide an opportunity for staff to participate in self-regulation. “Petrifilm plates are a convenient, simple-to-use measuring stick, ideal for testing by our field operators and in the plant,” Mulligan added.

For those concerned about the idea of eating one of Australia’s national emblems, you should know that the kangaroo population at 35 million animals is significantly higher today



than when the country was first settled by the British in 1788. Kangaroo meat has excellent nutritional value, being rich in protein and iron and low in both cholesterol and saturated fats. Southern Game Meats’ supply is licensed and strictly supervised as part of the government’s balanced land and kangaroo management program.



3M Microbiology Products, Japan

3M Works Overtime in Response to Japan Food Poisoning

3M Microbiology Products in Japan and the U.S. operated in high gear last year to meet the demand for increased food product testing triggered by the outbreak of Hemorrhagic *E. coli* O157:H7 in Japan. Takatoshi Moriyama, 3M Japan Microbiology

supervisor, worked closely with the Japan Ministry of Health and Welfare and major food processors during the epidemic. 3M™ Petrifilm™ Test Kit-HEC is one of the approved methods used by the Japanese government to identify *E. coli* O157:H7.

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Focus on Microbial Testing as Sanitation SOP

The emphasis on microbial testing means that sanitation SOP's have evolved beyond washing floors and wearing hairnets. The new regulation makes *E. coli* testing an integral step in the process of preparing safe, high-quality meat and poultry products.

While the first phases of Mega Reg implementation began in January, USDA enforcement will not begin for a number of months—giving processors time to refine their systems and create baseline measurements for future comparison. Following this grace period, Food Safety and Inspection Service (FSIS) officials will begin inspecting products both visually and on a microscopic level.

Sampling the Carcass

Under the requirements for *E. coli* testing, processors must collect samples from the carcass, test the samples and maintain records of the results. They must also document who is authorized to collect samples, sample locations, how randomness is achieved and the steps being taken to ensure sample integrity.

Regardless of whether *E. coli* testing is performed in-house or by an outside lab, carcass sampling is the first and often most time-consuming step in the process.

Using cattle as an example, let's review the sampling, testing and recording process. (See Table 1 for more information about requirements for testing cattle, swine, chicken and turkey.) The USDA requires operations processing more than 6,000 head of cattle per year to test one out of every 300 carcasses for *E. coli*. Samples should be taken from carcasses that have been chilled 12 or more hours after slaughter and tested as soon as possible. Sampling can be done by using the excise method or the sponge method.

The sponge sampling process for cattle begins by hydrating a sponge in a Whirl-Pak® bag with 10mL of sterile Butterfield's Phosphate Diluent (BPD). Using the same sponge, three 100cm² areas are sampled: the flank, brisket and rump. After returning the sponge to sample bag, 15 more mL of BPD is added to bring the bag's volume to 25mL. Once this process is completed, the sample is ready for testing.

Comparing the Options: On-site vs. Off-site Testing

Processors have two choices for microbial testing: they can test on-site using AOAC-approved methods or send samples to an outside laboratory, which must analyze the sample no later than the day after collection.

Some smaller operations have been reluctant to try testing on their own, fearing the costs involved with setting up an in-house laboratory—including lab equipment, storage space, and additional labor. But simpler, faster and more precise testing methods have made on-site microbial testing a cost-effective and time-saving option. In reality, *E. coli* testing is easier than

most people think—and it doesn't require a degree in microbiology. Using an AOAC® Official MethodSM, most processors are able to test quickly and efficiently using minimal equipment and staff time.

Perhaps the most compelling case for in-house testing involves access to and control of your plant's *E. coli* data. Hands-on testing provides quicker results, reducing response time if irregularities appear. Many processors prefer the security of performing their own tests and analyzing results internally—giving them the ability to control their program from start to finish.

Testing Samples On-site: The Process

Keep in mind that sampling the carcass is the most difficult part of *E. coli* testing and must be done on-site. Once sampling is completed, on-site testing with Petrifilm plates requires only three steps—inoculate the plates with the sample, incubate the plates

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Table 1

<i>E. coli</i> Testing Using Petrifilm <i>E. coli</i> Count Plates				
	Cattle	Swine	Chicken	Turkey
Frequency¹	1/300	1/1000	1/22,000	1/3000
Sample	Carcass	Carcass	Carcass	Carcass
Sample Dilution	Excise ² 25mL	Excise ² 25mL	Bird/ 400mL	Bird/ 600mL
Sample Plating				
Plate #1 (duplicates)	1mL	1mL ³	1mL	1mL ⁴
Plate #2 (duplicates)	1mL of 1/10	1mL of 1/1000	1mL of 1/10	
Counts on PEC⁵				
Acceptable	negative growth	≤10	≤100	FSIS to Advise
Marginal	<100	>10 ≤10,000	>100 but ≤100 (1/10)	
Unacceptable	≥100	>10,000	>100 (1/10)	

Footnotes

¹ Except for Very Low Volume (VLV) establishments:
Cattle - <6000 head per year
Swine - <20,000 head per year
Chicken - <440,000 birds per year
Turkey - <60,000 birds per year
VLV must test one sample per week until at least 13 test results have been obtained.
VLV establishments must test 13 samples/yr between June through August.

² These results are from the January 1994 baseline study. A new baseline is now underway.

³ Consider additional Plate #3 at 1/100 dilution to insure a reportable number in marginal region.

⁴ FSIS baseline study has not been completed.

⁵ Each 1mL of initial sample represents 12cm² (300cm² sampled with 25mL sterile diluent).

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and read the results. With Petrifilm plates, lab equipment is minimal. (See *Introducing The Basic Essentials For Microbiology Testing* on the cover.)

To continue with the cattle example, an in-house testing program using Petrifilm plates would require inoculating two plates with the sample: one undiluted, and one 1:10 dilution. The USDA also recommends running one duplicate of each plate.

For the direct (undiluted) plate, remove one milliliter of sample from the sample bag and inoculate onto the Petrifilm plate. Repeat the process for the duplicate plate. To make the diluted sample, place one mL of sample into nine mLs of BPD solution. Then plate one mL from that mixture; repeat the process for the duplicate plate.

The total coliform count consists of both the red and blue colonies associated with gas at 24 hr. Reincubate plates an additional 24 ± 2 hr. to detect any additional *E. coli* growth. Regardless of incubation time, whenever a blue colony associated with gas appears, it is a confirmed *E. coli*. *E. coli* colonies can be counted easily and precisely using the built-in grid on the Petrifilm plates.

Recording the Results

Begin by counting any *E. coli* colonies on the direct sample plates. For our cattle example, negative *E. coli* colony growth must be observed to report an acceptable result according to USDA standards. (See Table 2.)

If any *E. coli* colony growth appears on the direct sampling plate, refer to the second set of plates prepared with the 1:10-diluted sample. (See Table 2.)

Table 2

Converting Petrifilm Plate Cattle Results to USDA Criteria		
Total sample size = 300cm ²	=	Diluted sample size = 12cm ²
Total diluent volume = 25mL	=	Sample volume = 1mL
25mL total diluent volume representing 300cm ² total sample area size means that 1mL of sample represents a 12cm ² area.		
Petrifilm plate count is divided by 12 to equal the count per cm ² as recommended by the USDA.		
Number of <i>E. coli</i> colony forming units		
Method	USDA Excise	Petrifilm Plates Sponge ¹
Acceptable	negative growth	negative growth
Marginal	<100/cm ²	positive growth ¹
Unacceptable	≥100/cm ²	

¹ The sponge method requires statistical process control procedures. FSIS to advise.

An unacceptable result should trigger immediate action to review the process controls, discover the cause of the problem and prevent it from reoccurring. Three or more marginal or unacceptable results in 13 consecutive tests also signal the need to review process controls—and may result in USDA action.

New Opportunities

It's clear that the next few months will be a time of change for both FSIS guidelines and processors as they work to comply with the Mega Reg, which continues to evolve. Many will find that simple and cost-effective methods have made on-site *E. coli* testing an easily implemented, realistic alternative to outsourcing—providing quicker results and more control over the testing program.

Helpful Hints

Tips for Using Petrifilm Plates

Sharing ideas for using a product can benefit everyone. That is why we have launched this ongoing column containing helpful tips for using 3M™ Petrifilm™ plate products. Many of these tips are inspired by questions or useful ideas that we are hearing directly from you.

- When using Petrifilm plates for environmental sampling be sure to use **thiosulfate-free letheen broth** if sanitizer may be present. Letheen broth neutralizes halogen, quaternary ammonium, and acid sanitizers. Do not use diluents containing thiosulfate or sodium citrate with Petrifilm plates.
- To ensure accurate results it may be necessary to adjust the pH of acidic foods before plating onto any of the Petrifilm

plates for bacterial enumeration. Refer to product literature for specific instructions.

- The interpretation of blue colonies without gas on Petrifilm™ *E. coli* Count plates varies by method.

AOAC® Official MethodsSM (991.14 Coliform and *Escherichia coli* Counts in Foods-Dry Rehydratable Film Methods) states that blue colonies without gas are not counted as *E. coli*.

The AFNOR method (3M 01/04-09/92) states that blue colonies without gas may be *E. coli* and should be confirmed if necessary.

It is important for each food processor to determine the significance of blue colonies without gas for its operation.



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