

Neogen® Petrifilm® *Bacillus cereus* Count Plate Performance Summary

Overview

The Neogen® Petrifilm® *Bacillus cereus* Count (BC) Plate is a selective and differential sample-ready-culture-medium system which contains proprietary nutrients, a cold-water-soluble gelling agent, chromogenic indicators and a lecithinase substrate that facilitates colony enumeration. This medium is used for the enumeration of the *Bacillus cereus* group, also known as the *B. cereus sensu lato* group, which are aerobic, spore forming, Gram positive rods.

The purpose of inclusivity and exclusivity testing is to ensure that the Petrifilm *Bacillus cereus* Count Plate can detect *Bacillus cereus* while discriminating from non-*Bacillus cereus* bacteria. Inclusivity is defined as the ability of a method to detect the target analyte from a wide range of strains. Exclusivity is defined as the lack of interference from a relevant range of non-target strains.

The purpose of the food study is to show performance in matrices of interest on Petrifilm *Bacillus cereus* Count Plate as compared to the ISO 7932 Method. The selected matrices may have been chosen because they present potential common reservoirs for *Bacillus* organisms and/or are regulated categories of interest. These matrices have a variety of background flora levels and are typically artificially inoculated for testing to assess recovery against a standard reference method.

Method – Inclusivity and Exclusivity Testing

Pure bacteria cultures were derived from purchased culture collection preparations or from frozen stock cultures. Inclusivity and exclusivity cultures were cultured in a non-selective growth medium and plated at a concentration within the countable range of the Petrifilm *Bacillus cereus* Count Plate. Organisms were tested according to the Petrifilm *Bacillus cereus* Count Plate product instructions and incubated at both 30°C and 35°C for 20-24 hours.

Results

Inclusivity

Of the 38 isolates tested, 38 were detected by Petrifilm *Bacillus cereus* Count Plate at both 30°C and 35°C.

Exclusivity

Of the 32 isolates tested, 32 of the isolates were inhibited, produced atypical colonies (blue colonies or pinpoint red colonies with no cream/white precipitate around the colony) or were not detected on the Petrifilm *Bacillus cereus* Count Plate at 30°C and 35°C. Those that produced atypical colonies include *Bacillus* species, *Enterococcus faecalis*, *Listeria* sp. and *Staphylococcus aureus*.

Table 1. Inclusivity List (n=38)

Organism	Organism
<i>Bacillus cereus</i> (25) ¹	<i>Bacillus pseudomycooides</i> (2)
<i>Bacillus cytotoxicus</i> (2)	<i>Bacillus thuringiensis</i> (2)
<i>Bacillus mobilis</i>	<i>Bacillus toyonensis</i>
<i>Bacillus mycooides</i>	<i>Bacillus weihenstephanensis</i>
<i>Bacillus pacificus</i>	<i>Bacillus wiedmannii</i>
<i>Bacillus paranthracis</i>	

*Strains were sourced from Neogen Food Safety Culture Collection, Cornell University Culture Collection, American Type Culture Collection (ATCC), and BEI Resources. See Reference 1.

Table 2. Exclusivity List (n=32)

Organism	Organism
<i>Bacillus atropheus</i>	<i>Klebsiella pneumonia</i>
<i>Bacillus badius</i>	<i>Lactobacillus lactis</i>
<i>Bacillus circulans</i>	<i>Listeria innocua</i>
<i>Bacillus coagulans</i>	<i>Listeria ivanovii</i>
<i>Bacillus licheniformis</i> (2)	<i>Listeria monocytogenes</i>
<i>Bacillus megaterium</i>	<i>Proteus mirabilis</i>
<i>Bacillus pumilus</i> (2)	<i>Proteus vulgaris</i>
<i>Bacillus subtilis</i> (2)	<i>Pseudomonas aeruginosa</i>
<i>Bacillus spizizenii</i>	<i>Serratia marcescens</i>
<i>Citrobacter koseri</i>	<i>Staphylococcus aureus</i> (2)
<i>Enterobacter amnigenus</i>	<i>Staphylococcus epidermidis</i>
<i>Enterococcus faecalis</i> (2)	<i>Staphylococcus schleiferi</i>
<i>Escherichia coli</i>	<i>Streptococcus bovis</i>
<i>Klebsiella oxytoca</i>	

* Strains were sourced from Neogen Food Safety Culture Collection and American Type Culture Collection (ATCC).

Food Study

Samples were prepared at a 1:10 dilution in Butterfield’s Phosphate Buffer and tested according to the Petrifilm *Bacillus cereus* Count Plate product instructions and incubated at both 30°C and 35°C for 20-24 hours. Additionally, samples were spread across MYP agar and incubated at 30°C for 18-48 hours following the ISO 7932 standard. Results from the Petrifilm *Bacillus cereus* Count Plate method were not statistically different from the reference method.

Table 3. Food Study Performance (n=120 foods tested)

Reference Method	Petrifilm <i>Bacillus cereus</i> Count Plate	Petrifilm Incubation Temp (±1°C)	Result (ANOVA P-Value)
ISO 7932 Medium: MYP agar – spread plate Diluent: Butterfield’s Phosphate Buffer Incubation Time: 18-48 hours Temperature: 30°C	Petrifilm BC Plate Diluent: Butterfield’s Phosphate Buffer Incubation Time: 20-24 hours	30°C	No statistical differences (0.093)
		35°C	No statistical differences (0.951)



Table 4. List of Foods Tested

Dried Food/Spices	Multi-Component Foods/RTE	Dairy	Baby Food	Poultry/Meat /Seafood	Alternative Protein	Pet Food
Rice Vermicelli	Rosemary Chicken Salad	Chunky Blue Cheese	Carrots and Peas	Crispy Tiny Shrimp	Quinoa Cowboy Veggie Burger	Wet Dog food Beef Tray
Yellow Corn Meal Masarepa	Garlic Pasta Salad	Spanish Flan Style Custard	Milk Drink	Honey Smoked Salmon	Vegetable Masala Burger w/Indian Spices	Dried Cat Food
Rice Flour	Pia Cake	Mousse Truffle	Cereal for Baby	Prepared Fish BBQ Sticks	Plant Based Hotdogs	
Rice Dumpling Flour	Cuttlefish Fried Rice Cake	Dairy Compound Powder Beverage	Infant Formula	Duck Mousseo	Plant Based Beef	
Flour Mix for Churros	Cheese filling Breadstick	Chèvre Goat Cheese	Infant formula w/Probiotics	Squid Shreds	Chickenless Crispy Tenders	
Mung Bean Flour	Spicy Tuna Roll	Monster Cookie Ice-cream		Skinless Atlantic Salmon	Plant Based Fish Fillets	
Chickpea Flour	Vegetable Hand Rolled Spring Roll	Raw Milk*		Squid Seafood Snack	Protein Shake	
Starch Flour	Egg Fettuccine pasta	Buttermilk Powder		Grilled Fajita Chicken Breast Strips	Powdered Peanut Butter	
Instant Corn Flour	Vegetable Fried Rice	Spanish Flan Style Custard		Uncured Genoa Salami	Barley Grass Juice Powder	
Custard Mix	Rotisserie-Seasoned Chicken Salad	Yogurt		Pork+Beef Uncured Pepperoni		
Herb Seasoned Classic Stuffing	Tomato Basil Soup with Pasta	Vanilla Caramel Pecan Ice Cream		Chicken Breast Fillets		
Pancake and Waffle Mix	Develed Egg Potato Salad	Soft Vanilla Ice Cream				
Cornbread Mix	Cheese Tortellini	Creamy Havarti				
Thin Mini Crackers	Instant Mashed Potatoes	Vanilla Bean Ice Cream				
Organic Couscous	Buttermilk Ranch	Mozzarella				
Rice Orzo Pilaf Mix	Organic Ranch Dressing	Reduced Fat Milk				
Tricolor quinoa	Caesar Dressing	Crème Fraiche				
Seasoning Mix for Noodle Soup	Ranch Dressing	Crème Brûlée				
Mushroom Seasoning	Rice Pudding	Triple Cream Brie Cheese				
Creole Seasoning		Dried Whole Milk				
Seafood Seasoning		Cheddar Cheese Sauce Mix				

*Raw milk with low background

Notes and References:

- The following reagents were obtained through BEI Resources, NIAID, NIH: *Bacillus cereus*, Strain Tor 16585, NR-12151; Strain VD115, NR-22148; *Bacillus cereus*, Strain VD148 NR-22150 ; Strain BAG1X1-1, NR-28575; Strain BAG1X2-1, NR-28578; Strain BAG10-2, NR-28582; Strain BAG60-1, NR-28593 ; Strain BAG4X2-1, NR-28599; Strain BAG50-1, NR-28605; Strain AND1407, NR-22159; Strain MSX-A1, NR-22166; Strain FDA 5, NR-608 ; Strain E33L, NR-12264; Strain NRS 201, NR-2488; Strain NRRL B-569, NR-2492; Strain Sneath 5/B (PCI 246), NR-2493; Strain NRRL B-569, NR-52260.
- ISO 6887, Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension, and decimal dilutions for microbiological examination.
- ISO 7932, Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *Bacillus cereus* - Colony-count technique at 30 degrees Celsius

