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Compatibility of 3M™ Petrifilm™ Plates with Neutralizing Buffered Peptone Water

Introduction

Antimicrobial chemical interventions or processing aids are used during poultry processing to reduce contamination of carcasses and parts by foodborne pathogens and reduce the risk of human foodborne illnesses. The pathogens of most concern in this industry belong to the genus *Salmonella* and *Campylobacter*. Control of these pathogens during processing is critical and is often under scrutiny by consumers and the media. In response to an article published in the Washington Post, the United States Department of Agriculture (USDA) reviewed the effectiveness of Buffered Peptone Water (BPW) to neutralize and prevent carry over of antimicrobial activity from active ingredients commonly used in the industry.[1] In a study conducted by Gamble et al.,[2] findings indicated that Buffered Peptone Water may not be able to fully reduce the effect of potential carryover sanitizers on poultry verification samples, which may lead to false-negative results during *Salmonella* testing. As a result of these studies, USDA Agricultural Research Service (ARS) developed a new neutralizing medium for whole bird and part rinses, and turkey carcass sponge swabs referred to as neutralizing Buffered Peptone Water (nBPW). USDA Food Safety and Inspection Service (FSIS) implemented the use of nBPW by inspection program personnel during verification sampling July 1, 2016.[3] While the use of nBPW is not required to be used by poultry producers for their own pathogen testing, producers might choose to rinse carcasses and parts in the nBPW for alignment with FSIS testing.

All of the published studies generated by USDA ARS are for whole bird and part tests for *Salmonella* and *Campylobacter* as it is the scope for the intended use of the medium. While sampling of whole birds and parts by poultry producers during processing is conducted routinely for pathogen testing, indicator testing is also a critical part of a monitoring or verification program. Indicator organisms tested for include aerobic (total or standard) plate count, *E. coli* and coliform count and *Enterobacteriaceae* counts. As these indicator organisms were not tested for in previous studies, there was a need for information on the compatibility of the nBPW formulation for commonly used indicator test methods including 3M™ Petrifilm™ Plates.

The formulation of nBPW includes several neutralizing components including thiosulfate, a high concentration of sodium bicarbonate and a final pH of 7.7 ± 0.5. It was unknown if these components at the given concentrations would have an impact on the 3M Petrifilm Plate methods. This is an important detail, considering the recommendations outlined in the product instructions for 3M Petrifilm Plates: Do not use diluents containing citrate, bisulfite or thiosulfate with 3M Petrifilm Plates; they can inhibit growth. To demonstrate the compatibility of nBPW with 3M Petrifilm Plates commonly used in the poultry processing industry, bacterial recovery on 3M Petrifilm Plates was compared to their respective agar reference method.

Materials and Methods

Media and Reagents

- 3M™ Flip-Top Dilution Bottle Buffered Peptone Water (90mL)
- Neutralizing Buffered Peptone Water (400mL)
- Bird Rinse Bags
- 5N Hydrochloric Acid (HCl)
- Standard Methods Agar
- Violet Red Bile Glucose Agar
- Violet Red Bile Lactose Agar with 4-Methyl-umbelliferyl-β-D-glucuronide (MUG)
- 3M™ Petrifilm™ Aerobic Count Plate
- 3M™ Petrifilm™ Rapid Aerobic Count Plate
- 3M™ Petrifilm™ *Enterobacteriaceae* Count Plate
- 3M™ Petrifilm™ *E. coli*/Coliform Count Plate

Whole bird and parts rinses were collected at three different manufacturing locations. Whole bird rinses were done both before and after invention while chicken wings were only rinsed after intervention. Treated birds and parts used in this study were treated with one of three types of interventions. The active ingredients in the three interventions were peroxyacetic acid, CPC or acid blend. Whole birds were allowed to drip for 1 minute before rinsing. Whole birds and chicken wings were rinsed in 400mL nBPW for 1 minute. Bird and part rinses collected held refrigerated before testing. Prior to testing, the pH of the bird rinse was measured and adjusted with 5N HCl, if needed, to between 6.6 to 7.2.

Standard Methods Agar (SMA), Violet Red Bile Glucose Agar (VRBG) and Violet Red Bile Lactose Agar (VRBL) with MUG were prepared according to the manufacturer's instructions. All agar media was tempered to 45°C prior to use. 3M Petrifilm Aerobic Count Plate, 3M Petrifilm Rapid Aerobic Count Plate, 3M Petrifilm *Enterobacteriaceae* Count Plate and 3M Petrifilm *E. coli*/Coliform Count Plate were brought to room temperature prior to use.

Bird and part rinse samples were serially diluted in BPW. Dilutions ranged from 1:1 to 1:10,000,000 and were plated on both the reference method and 3M Petrifilm Plates. Plates were incubated according to the conditions prescribed in the reference method or product instructions (see Table 1).

Table 1. Method Incubation Conditions.

Medium	Incubation Time	Incubation Temperature
Standard Methods Agar	48 hours ± 3 hours	35°C
Violet Red Bile Glucose Agar	24 hours ± 2 hours	37°C
Violet Red Bile Lactose Agar with 4-Methyl-umbelliferyl-β-D-glucuronide	24 hours ± 2 hours	35°C
3M Petrifilm Aerobic Count Plate	48 hours ± 3 hours	35°C
3M Petrifilm Rapid Aerobic Count Plate	24 hours ± 2 hours	35°C
3M Petrifilm *Enterobacteriaceae* Count Plate	24 hours ± 2 hours	37°C
3M Petrifilm *E. coli*/Coliform Count Plate	24 hours ± 2 hours	35°C

Following the appropriate incubation time, plates were removed from incubators and enumerated according to the reference method instructions or product instructions. Colony Forming Units (CFU)/mL of rinse were determined for all methods.

Results and Discussion

Data from either the reference method or 3M Petrifilm Plate with fewer than 15 colonies on the lowest dilution were removed from the statistical analysis. Counts were log transformed prior to analysis.

The number of total bacteria recovered on 3M Petrifilm Aerobic Count Plate and 3M Petrifilm Rapid Aerobic Count Plate from birds and parts rinsed in nBPW was not statistically different than the number recovered on SMA. The mean log difference between SMA and 3M Petrifilm Aerobic Count Plate was 0.14. The mean log difference between counts obtained from SMA and 3M Petrifilm Rapid Aerobic Count Plate was -0.02.

The number of *Enterobacteriaceae* bacteria recovered on 3M Petrifilm *Enterobacteriaceae* Count Plate was statistically different than the number recovered on VRBG. However, the number of bacteria recovered on 3M Petrifilm *Enterobacteriaceae* Count Plate was greater than VRBG by 0.18 Log.

The number of total coliforms recovered on the 3M Petrifilm *E. coli*/Coliform Count Plate was statistically different than the number recovered on VRBL with MUG. However, the number of total coliform colonies was greater on 3M Petrifilm *E. coli*/Coliform Count Plate by 0.07 Log.

The number of *E. coli* recovered on 3M Petrifilm *E. coli*/Coliform Count Plate was statistically equivalent to VRBL with MUG. Additionally, the mean log difference between the two methods was 0.08 Log.

Table 2. Paired t-Test and Mean Log Difference Results.

Methods (n=number of samples)	p-Value (95% CI)	Mean Log Difference
3M Petrifilm Aerobic Count Plate as compared to Standard Methods Agar (n=39)	0.283	0.14
3M Petrifilm Rapid Aerobic Count Plate as compared to Standard Methods Agar (n=34)	0.296	-0.02
3M Petrifilm *Enterobacteriaceae* Count Plate as compared to Violet Red Bile Lactose Agar (n=21)	0.000	0.18
3M Petrifilm *E. coli*/Coliform Count Plate for total coliform count as compared to Violet Red Bile Lactose Agar with 4-Methyl-umbelliferyl-β-D-glucuronide (n=17)	0.011	0.07
3M Petrifilm *E. coli*/Coliform Count Plate for *E. coli* count as compared to Violet Red Bile Lactose Agar with 4-Methyl-umbelliferyl-β-D-glucuronide (n=9)	0.074	0.08

In summary, the data has demonstrated that use of 3M Petrifilm Plates with nBPW for whole bird or part rinses provide statistically equivalent or better results than their comparative agar method. It is important to note that the success of these results was made possible by adjustment of the rinse pH using HCl to be within the recommended range for each method.

References

1. Hinton, A. Studies on Chemical Sanitizer Carryover in Poultry Processing [Presentation] USDA ARS. February 3, 2016.
2. Gamble GR et. al., Effect of Simulated Sanitizer Carryover on Recovery of *Salmonella* from Broiler Carcass Rinsates. *J Food Prot.* 2016 May;79(5):710–4.
3. FSIS Notice 41–16. New Neutralizing Buffered Peptone Water to Replace Current Buffered Peptone Water for Poultry Verification Sampling.

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