Indicator Organism and Pathogen Quantification: The Impact of Testing Methods & Interventions within a Poultry Facility

Introduction

About This Study

Both physical and chemical interventions are heavily used in the poultry industry to reduce *Salmonella* and *Campylobacter* presence on final poultry products driven by the USDA-FSIS (USDA Food Safety and Inspection Service) performance standards (PS). Challenges in determining intervention efficacy arise as prevalence testing only provides a part of the story, the other part told by quantification. Therefore, quantification-based baseline evaluations can assist in determining the full story of intervention efficacy and process control.

A study was completed to develop an indicator organism and pathogen baseline, with and without chemical interventions. By bio-mapping the processing chain from flock to final product of a large USDA inspected poultry processor, results demonstrate the value of rapid enumeration testing for microorganisms during live production and processing while determining the best practices and systems for quantification of microorganisms.

Equipment, Supplies and Reagents

- 24 oz Filtered Whirl-Pak® Bags
- Buffered Peptone Water (BPW)
- Neutralizing BPW (nBPW)
- Pipette and pipette tips
- MicroSnap™ Total (Total Viable Count)
- MicroSnap™ EB (*Enterobacteriaceae*)
- TEMPO® AC (Aerobic Count)
- TEMPO® EB (*Enterobacteriaceae*)
- BAX® MP Media
- BAX® Quant Solution
- BPW / Neutralizing BPW
- BAX® System Real Time *Salmonella*
- BAX® System SalQuant™

Methods

Sample Collection

Five poultry samples were collected at each poultry sampling location (at a large poultry processing facility), with and without interventions (treatment with various chemicals), over a period of 5 days (n = 450).

Sampling locations included:

- boot swabs
- live receiving
- rehang
- post evisceration
- post chill
- skin-on thighs
- skinless thigh
- wing parts
- ground wings

Enrichment

All samples were prepared from a single enrichment source; BPW was used for boot swabs and ground product and neutralizing BPW (nBPW; 400 mL) was used for poultry parts/carcass rinsates. For the presence of indicator organisms (Total Viable Count and *Enterobacteriaceae*), enrichments were tested with Hygiena™ MicroSnap™ and bioMérieux® TEMPO®. For *Salmonella* prevalence and enumeration, 30 mL of sample (in BPW/NBPW) was mixed with 30 mL of BAX MP + BAX Quant Solution and incubated at 42°C for 6 - 24 hours: incubation time was 6 - 10 hours for SalQuant (dependent on matrix: 6 hours for rinsates, 8 hours for ground product, and 10 hours for boot swabs) and 18 - 24 hours for prevalence, both using BAX System Real Time PCR Assay for *Salmonella*. At the indicated timepoints, samples were

tested with BAX System Real-Time *Salmonella* following established protocols. Resulting bacterial counts from SalQuant, MicroSnap, and TEMPO were converted to Log^{10} CFU/carcass (rinsate) or Log^{10} CFU/mL with comparisons using an ANOVA in JMP® with significance at $P < 0.05$.

Results & Discussion

Comparison of Pathogen & Indicator Organisms

Data was collected for the presence of indicator organisms in both no intervention and intervention samples using MicroSnap and TEMPO for TVC and EB. As shown in Figure 1, intervention had no effect on the sample population (P=0.167) indicating that physical interventions were just as effective as chemical interventions at reducing indicator organisms and pathogen numbers. This was true for both TVC results (Figure 1) as well as EB counts (Figure 2). Sampling day had no effect on results either.

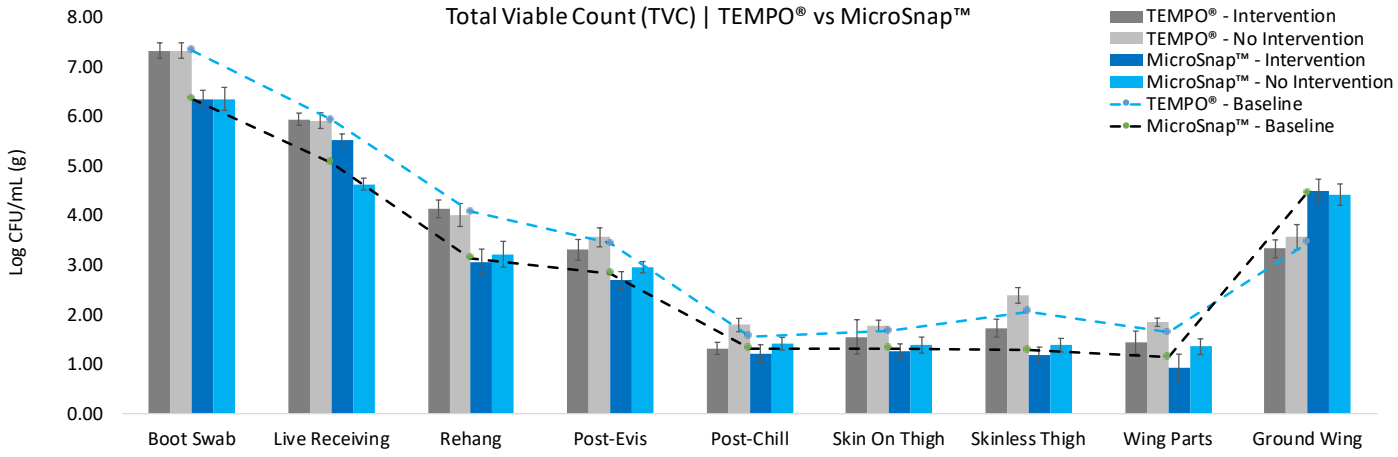


Figure 1. Total Viable Counts (TVC; Log CFU/mL(g)) from flock to final product in a large USDA inspected poultry processing facility utilizing Hygiena MicroSnap and bioMérieux TEMPO.

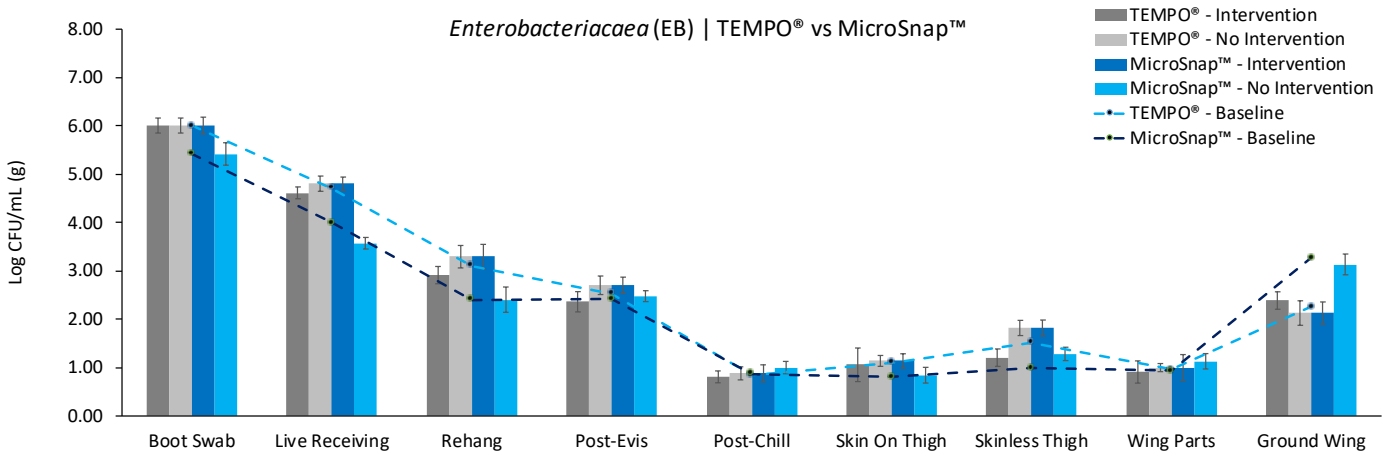


Figure 2. *Enterobacteriaceae* counts (EB; Log CFU/mL(g)) from flock to final product in a large USDA inspected poultry processing facility utilizing Hygiena MicroSnap and bioMérieux TEMPO.

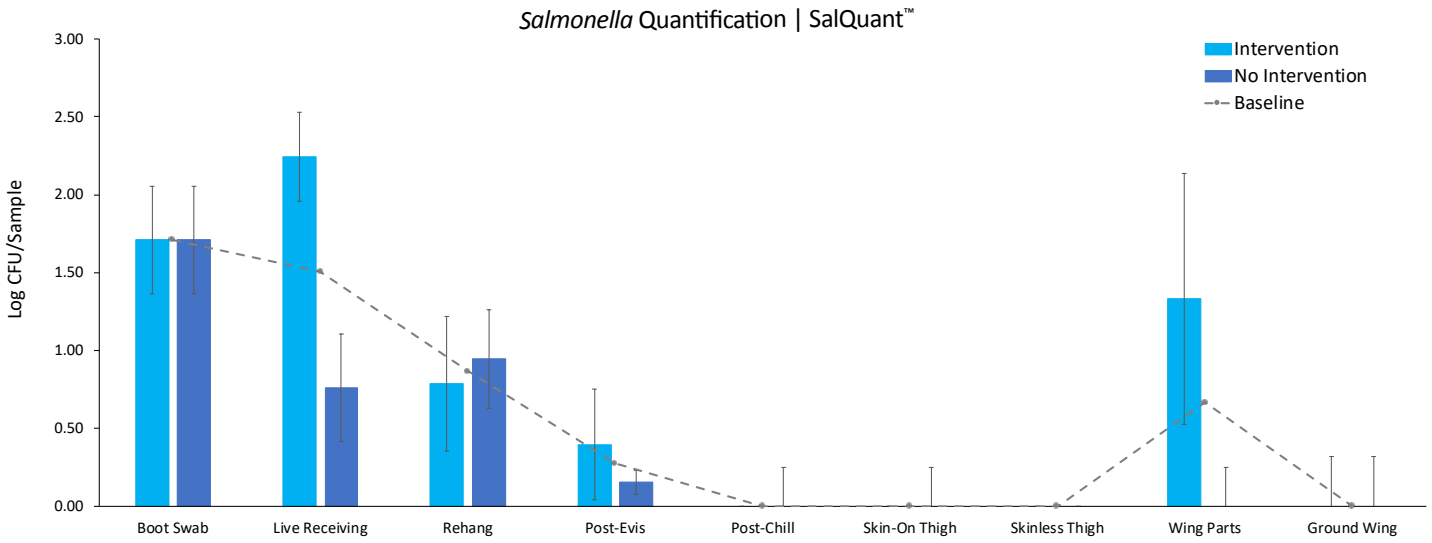


Figure 3. *Salmonella* Quantification (Log CFU/Sample) from flock to final product in a large USDA inspected poultry processing facility utilizing Hygiena SalQuant.

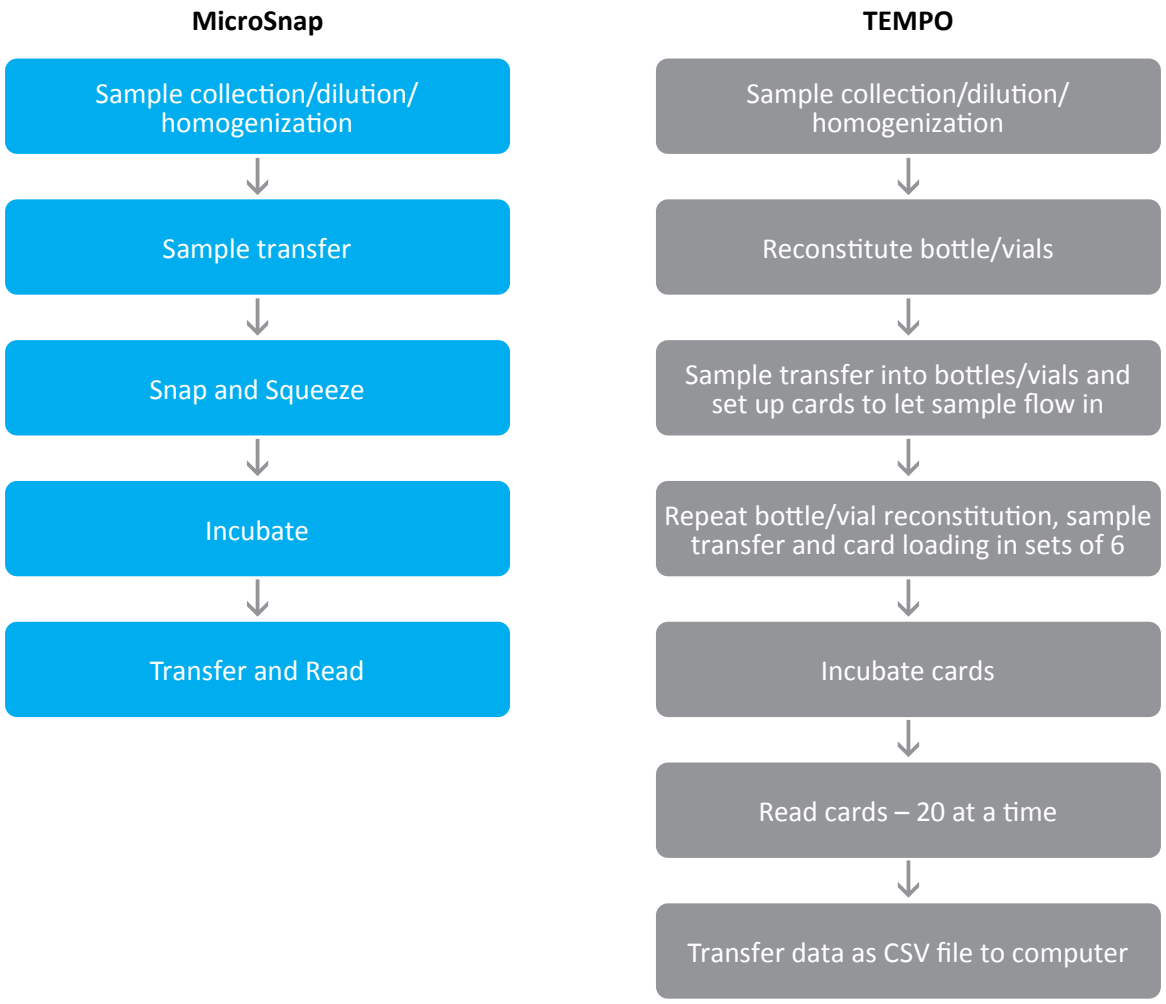
As shown in Figures 1 and 2, indicator organism counts decreased from boot swabs samples to initial live receiving samples, though no interventions, chemical or physical were applied. However, *Salmonella* counts increased by 0.5 Log^{10} CFU/carcass from boot swab samples to live receiving samples (Figure 3). Even though no interventions were applied, 'no intervention' birds were sampled first after the carcass was hung on a wall, causing potential contamination to intervention samples. Post live receiving, both pathogens and indicator organism counts continued to decrease, regardless of intervention, until further processing where ground wing samples rebounded for prevalence and quantification. (Note: For *Salmonella*, wing parts were only positive on the first day, which may have been due to cross-contamination).

MicroSnap vs TEMPO Use for Indicator Organism Detection

When determining what indicator organism testing method to select, many variables must be considered. The primary factor should be consistent, accurate performance. The data here demonstrates this accuracy – as MicroSnap detection levels were confirmed by PCR. In addition, the results show that both MicroSnap and TEMPO indicator organism tests provide similar quantifiable results in all cases analyzed (Fig 1 and 2); however, MicroSnap has the advantage when it comes to the time to results and ease of use (See Table 1). MicroSnap can provide results in less than one shift (6 - 7 hours), while TEMPO results take days. In addition, when comparing the protocols for the two methods (see Figure 4), it is clear that MicroSnap is easier to use than TEMPO, especially for large sample numbers (TEMPO can only fill 6 cards (6 samples) at a time and after incubation, can only read 20 cards at a time while MicroSnap is a self-contained system for each sample, easily and rapidly readable in a Hygiena luminometer). Additionally, MicroSnap cultures can be further quantitated using SalQuant on the BAX System. When evaluating what system to use, one should consider all these factors (summarized in Table 1; visualized in Figure 4).

Table 1: Comparison of Methods for Organism Detection			
Consideration Factor	**MicroSnap**	**TEMPO**	**Traditional Plating**
Result Consistency/ Accuracy	Yes	Yes	Yes, fairly accurate if multiple plates are counted and averaged
Sample Processing Time (n=90)	1 hour	3 hours	Varies depending on plate numbers per sample
Incubation Time	6 – 7 hours	24 – 48 hours	24 – 48 hours or longer
Read Time	80 minutes	30 minutes, if batched	Varies depending on plate numbers per sample (>80 minutes)
TOTAL TIME	**8 – 9 hours**	**27 – 51 hours**	**27+ hours**

Figure 4: Comparison of MicroSnap and TEMPO Workflows



Conclusions

Overall, this study provides evidence that physical interventions are just as effective as chemical interventions at reducing indicator organisms and continually reduce pathogen counts to safe levels. These conclusions can further promote reducing chemical usage while still producing a safe and wholesome product.

Furthermore, this study demonstrates that indicator organism testing, using MicroSnap, provides trending data supporting the actual levels of pathogens present. As indicator organism levels rise and fall, pathogen (*Salmonella*) levels fluctuate in parallel. Results also demonstrate the equivalency of MicroSnap test devices and TEMPO tests in detecting indicator organism levels and the advantages of MicroSnap over TEMPO: faster time to results – 8 - 9 hours vs 27 - 51 hours and ease of use – fewer and simpler steps for MicroSnap. Moreover, TEMPO does not have an enumeration test for *Salmonella*, so pairing MicroSnap with BAX System SalQuant analysis provides lower enumeration limits and accurate results. Therefore, MicroSnap is a more viable alternative as it provides software-based capabilities that can be paired with SalQuant using the Hygiena system for rapid, accurate results and enumeration in a single day.

References

1. TEMPO® is a registered trademark of bioMérieux
2. MicroSnap™ is a registered trademark of Hygiena™
3. BAX® is a registered trademark of Hygiena™