

Accuracy and specificity of novel DNA Signature Capturing technology for pathogen detection in poultry processing

SYNOPSIS

In 2016, *Salmonella* and *Campylobacter* were the leading causes of foodborne diseases according to data published by the Centers for Disease Control and Prevention (CDC, Press Release, April 20, 2017). Accurate and reliable detection of these pathogens during food processing is critical in order to prevent entry into the food supply.

A comparative study was conducted at a poultry processing facility to assess the performance of two rapid methods, Veriflow® and an antibody-based platform, for the detection of *Salmonella* and *Campylobacter* species in raw poultry parts. Discrepant results from the two methods were analyzed by USDA-FSIS MLG Reference methods. False positive and false negative rates, as well as time to results, were assessed. Veriflow did not produce any false positive or false negative results for either pathogen. Whereas, the antibody-based method did not detect any of the 7 positive results in the *Salmonella* study, and resulted in an 18.75% false positive and 25% false negative rate in the *Campylobacter* study. In conclusion, Veriflow proved to be a more accurate and user-friendly rapid diagnostic method for the detection of *Salmonella* and *Campylobacter* in raw poultry samples.

OVERVIEW

An AOAC-RI approved technology for detection of pathogens and spoilage organisms in food processing is gaining attention from poultry processors seeking a rapid method that improves accuracy and is easy to deploy on-site. Veriflow, developed by Invisible Sentinel, features DNA Signature Capturing technology - a novel method for DNA amplification and identification coupled with a simple, unencumbered method for visualization and data interpretation.

Study methods

The aim of this study was to validate the performance of Veriflow technology. A poultry producer conducted a side-by-side evaluation of the Veriflow system with a well known antibody-based, automated system in place at their processing facility. Matched samples were split for detection of *Salmonella* spp. and *Campylobacter* spp. respectively using the Veriflow technology and the antibody-based assay.

Twenty raw poultry products at various stages of processing were collected at the facility. Products included whole birds, rehangs, post-chill birds, and leg quarters. To prepare the samples, the poultry products were rinsed with 400 mL of Buffered Peptone Water (BPW) in a sealed bag and agitated thoroughly for 2 minutes. Following the agitation, samples were split for the detection of *Salmonella* spp. and *Campylobacter* spp. using the two systems (Fig. 1).

I. SALMONELLA SPECIES

a. Analysis of samples by Veriflow *Salmonella* species (SS) Method

Following agitation, 30 mL of poultry rinsate was transferred to 36 mL of pre-warmed (35°C) Invisible Sentinel (IS) chicken rinse enrichment broth containing novobiocin (2 mg/mL) and homogenized for 2 minutes. The samples were incubated at 35°C for 18 h. One milliliter of enriched sample was heat inactivated and cooled to room temperature. After cooling, 5 µL of sample was transferred into a PCR reagent tube and cycled in a thermocycler. Tubes were removed from thermocycler and 4 drops of provided buffer was directly added to each PCR tube. The entire contents of the PCR tube were transferred into a Veriflow SS Assay cassette sample window. After 2 min, 4 drops of provided buffer were added directly to each Veriflow SS Assay cassette sample window. After an additional 1 min, the switch was retracted and results observed in sample window were recorded, with one line indicating negative and 2 lines indicating positive.

b. Analysis of samples by Antibody-based *Salmonella* species Method

The poultry rinses were transferred to pre-measured BPW media and homogenized for 2 minutes. Following the addition of a *Salmonella* selective supplement, the samples were incubated for 18-24 h at the specified temperature. An aliquot of the enriched sample was then transferred into the wells of the testing device and heat inactivated using a specially designed heat block for a specified time. A pipetting device for each sample was loaded onto the instrument followed by the testing device. The instrument was run according to the manufacturer's instructions and the results were visualized on a computer using the manufacturer's proprietary software program.

c. Confirmatory analyses

Samples in which discrepancies were observed between the two methods (n=7), were further analyzed for *Salmonella* spp. following the procedures outlined in the USDA/FSIS MLG reference method 4.09.

II. CAMPYLOBACTER SPECIES

a. Analysis of samples by Veriflow *Campylobacter* species Method

Following agitation, 27 mL of poultry rinsate was transferred to a 50 mL conical and enriched with 27 mL of IS Bolton Broth and double selective supplement. The samples were incubated for 4 hours at 37°C and then re-incubated at 42 ± 1°C for 20 hours. Five hundred microliters of enriched sample was heat inactivated and cooled to room temperature. After cooling, 5 µL of sample was transferred into a PCR reagent tube and cycled in a thermocycler. Tubes were removed from thermocycler and 4 drops of provided buffer was directly added to each PCR tube. The entire contents of the PCR tube were transferred into a Veriflow *Campylobacter* Assay cassette sample window. After 2 min, 4 drops of provided buffer were added directly to each Veriflow *Campylobacter* cassette sample window. After an additional 1 min, the switch was retracted and results observed in sample window were recorded, with one line indicating negative and 2 lines indicating positive.

b. Analysis of samples by Antibody-based *Campylobacter* species Method

The poultry rinses were transferred to pre-measured proprietary *Campylobacter* broth and homogenized for 2 minutes. Samples were then transferred and incubated in a microaerophilic environment for 44-52 h at 42°C. An aliquot of the enriched sample was then transferred into the wells of the testing device and heat inactivated using a specially designed heat block for a specified time. A pipetting device for each sample was loaded onto the instrument followed by the testing device. The instrument was run according to the manufacturer's instructions and the results were visualized on a computer using the manufacturer's proprietary software program.

c. Confirmatory analyses

Samples in which discrepancies were observed between the two methods (n=4), were further analyzed for *Campylobacter* spp. following the procedures outlined in the USDA/FSIS MLG reference method 41.04.

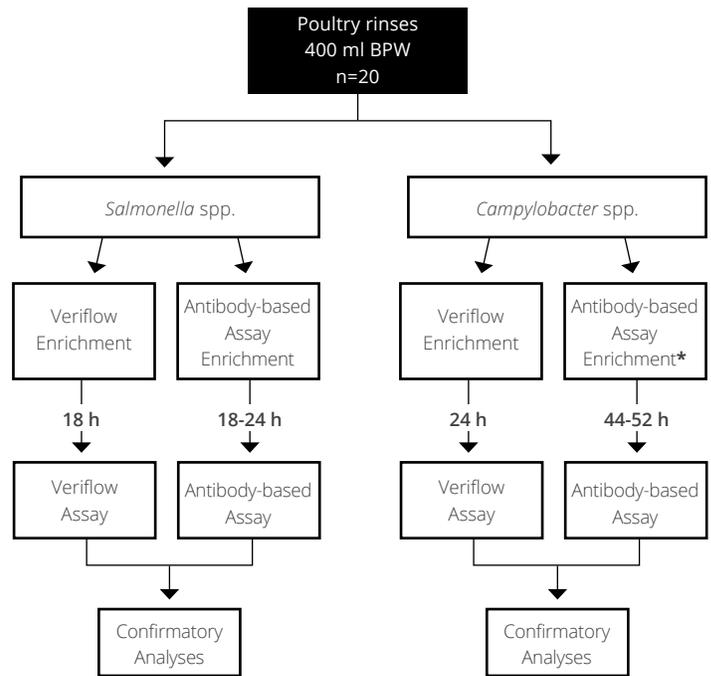


Fig.1 Study Design

*Enrichment performed under microaerophilic conditions

RESULTS

I. SALMONELLA SPECIES

In the study of *Salmonella* spp., 7 samples were presumptive positive by the Veriflow SS method, however all 20 samples were found to be negative by the antibody-based method (Fig. 2). All seven positive samples were culturally confirmed following the FSIS Microbiology Laboratory Guidebook (MLG) 4.09. The antibody-based method thus resulted in a 100% false negative rate (Fig. 4) in the 7 samples that were confirmed positive by the reference method.

The time to results for both detection methods (Table 1) was comparable (<24 h). Conventional methods require at least 6 days to confirm a positive result.

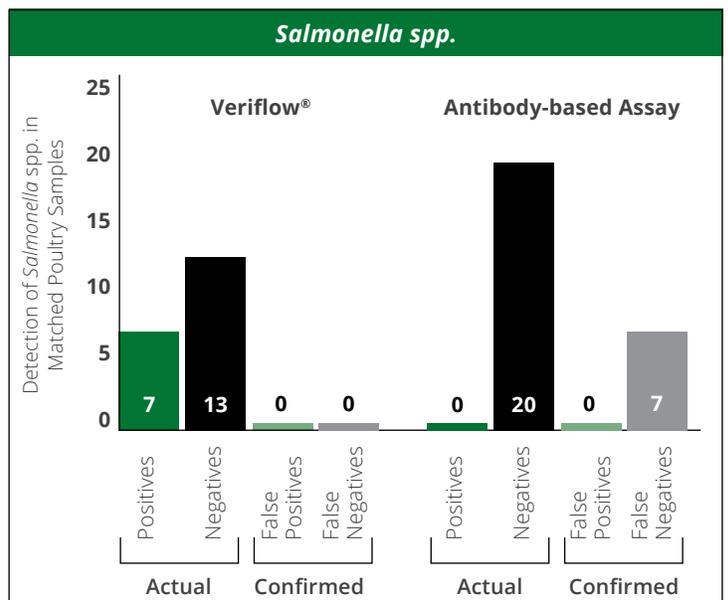


Fig. 2 Summary of results by Veriflow® *Salmonella* spp. method versus the antibody-based method

II. CAMPYLOBACTER SPECIES

For *Campylobacter* spp., results of the study demonstrate that the Veriflow *Campylobacter* spp. method reported 4 positives and the antibody-based method reported 6 positives. The Veriflow method reported 16 negatives and the antibody-based method reported 14 negatives. (Fig. 3). Discrepancies between the two methods (n = 4 samples) were culturally confirmed following the using FSIS Microbiology Laboratory Guidebook (MLG) 41.04. Upon analysis of the discrepancies between the two methods, an 18.75% false positive and a 25% false negative rate was observed with the antibody-based method (Fig. 4). For every discrepant result, the reference method confirmed the Veriflow result to be accurate.

The time to results for both detection methods were significantly different (Table 2). The enrichment time involved in the Veriflow *Campylobacter* spp. method requires a total of 24 h. In comparison, the antibody-based method not only requires a minimum of 52 h of enrichment time, which is 28 h longer than the Veriflow method, but also requires incubation under microaerophilic conditions to support pathogen recovery and growth. Conventional methods require at least 6 days to confirm a positive result.

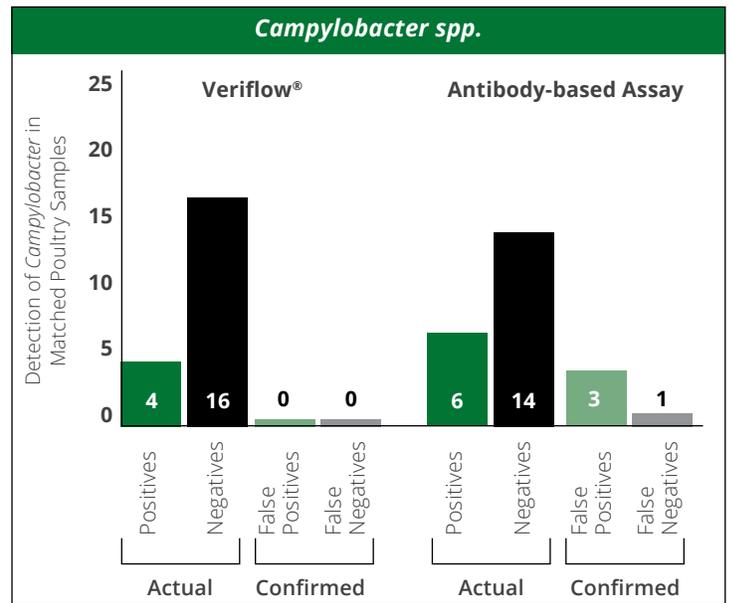


Fig. 3 Summary of results reported by Veriflow® *Campylobacter* spp. method versus the antibody-based method

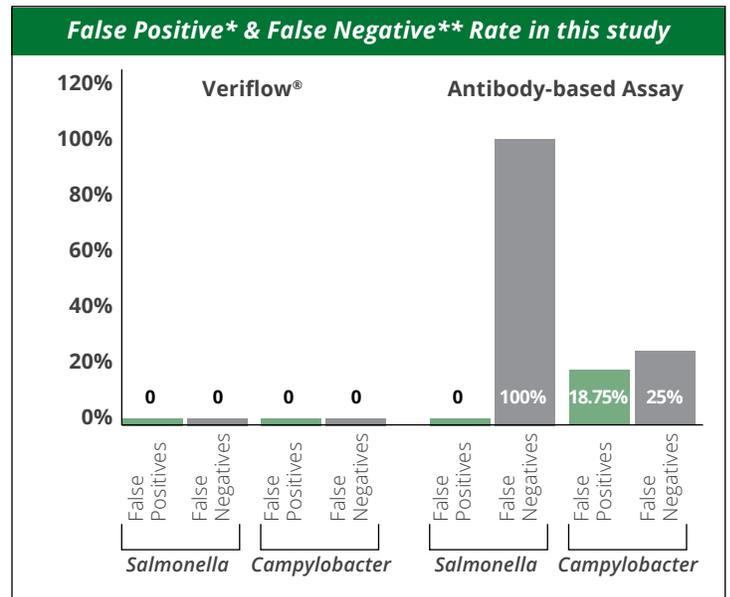


Fig 4. False positive and False negative rate observed in Veriflow versus the antibody-based assay used for *Salmonella* spp. and *Campylobacter* spp. as compared to confirmed culture results

*False Positive Rate is defined as the percentage of true negative samples that incorrectly received a positive test result.

**False Negative Rate is defined as the percentage of true positive samples that incorrectly received a negative test result.

SALMONELLA ASSAY	ACTIVITY	ANALYSIS TIME	TOTAL TIME
Veriflow® SS Method	Negative/Presumptive	IS Selective enrichment	18 h
	Positive Result	Sample Preparation, PCR & Cassette Analysis	<3 h
Antibody-based Method	Negative/Presumptive	Selective enrichment	24 h
	Positive Result	Sample Preparation & Sample Analysis	<1 h
	Negative Result	Pre-enrichment (non-selective)	24 h
		Selective enrichment	24 h
Selective and differential plating		24 h	
Reference Method	Positive Result	Pre-enrichment (non-selective)	24 h
		Selective enrichment	24 h
		Selective and differential plating	24 h
		TSI & LIA Culture/Serological Testing	24 h
		Selective plating for purity	24 h
		Biochemical Identification	24 h

Table 1. Description of the analysis time for identification of *Salmonella* spp.

CAMPYLOBACTER ASSAY		ACTIVITY	ANALYSIS TIME	TOTAL TIME
Veriflow® Campylobacter Method	Negative/Presumptive	IS Pre-enrichment	4 h	>1 day
	Positive Result	IS Selective enrichment	20 h	
		Sample Preparation, PCR & Cassette Analysis	<3 h	
Antibody-based Method	Negative/Presumptive	Selective enrichment	52 h	>2 days
	Positive Result	Sample Preparation & Sample Analysis	<1 h	
Reference Method	Negative Result	Primary Enrichment	48 h	4 days
		PCR & Selective plating	48 h	
	Positive Result	Primary Enrichment	48 h	6 days
		PCR & Selective plating	48 h	
Microscopic Exam & Latex Agglutination	24 h			
PCR Analysis	24 h			

Table 2. Description of the analysis time for identification of *Campylobacter* spp.

DISCUSSION

The Veriflow system contains a combination of proprietary enzymes and buffers to amplify and detect the genetic signature of target organisms and is resistant to inhibitors commonly found in poultry samples. This completely eliminates DNA extraction from the workflow and enables users to run the test with minimal sample preparation and one-step enrichment. With a goal to minimize any technical challenges associated with molecular-based methods, Veriflow was developed to deliver the highest level of accuracy while streamlining workflow in the facility and the laboratory. Additionally, Veriflow makes on-site PCR accessible and affordable with modest capital equipment requirements and hands-on time.

The results of this study demonstrated the accuracy, reliability, and ease of use of the Veriflow *Salmonella* and *Campylobacter* assays as compared to an antibody-based system for the detection of *Salmonella* and *Campylobacter* species in poultry rinsates. All positive and discrepant results were verified by the FSIS MLG reference method. Out of 20 poultry rinsates, the Veriflow method correctly detected 7 positive samples in the *Salmonella* assessment and 4 positive samples in the *Campylobacter* study. Markedly, no false positive or false negative results were observed in the entirety of the study using the Veriflow method. However, the paired analysis by the antibody-based assay demonstrated a 100% false negative rate (none of the 7 confirmed positive samples were detected) for the *Salmonella* assessment and a 25% false negative rate for the *Campylobacter* assessment (1 out of 4 positive samples

was not detected). Moreover, the antibody-based assay also showed an 18.75% rate of false positives during the *Campylobacter* assessment, where 3 out of the confirmed 16 negative samples caused a positive result.

CONCLUSION

The study demonstrates that Veriflow is not only an effective method for the detection of both foodborne pathogens found in poultry products but also a faster method that performs as an excellent screening method. The antibody-based assay suffered from high false positives and false negatives possibly due to cross-reactivity with antigens closely related to the *Salmonella* and *Campylobacter* spp. detected in the poultry samples. A 'built-in' microaerophilic environment for *Campylobacter* spp. enrichment in the Veriflow assay eliminates the need to purchase supplementary materials thereby reducing workflow complexity and cost per test. Overall, the Veriflow technology was found to be a more reliable and highly accurate rapid method giving producers the actionable data they need to manage the safety of their products.

DISCLOSURE

This study was performed at an independent site. Invisible Sentinel acknowledges the limited nature of this data however results are representative of field performance and validation studies. For additional information, please contact info@invisiblesentinel.com.

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