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PEER-REVIEWED ARTICLE

Food Protection Trends, Vol 45, No. 4, p. 257–267
https://doi.org/10.4315/FPT-24-042

Improving Enrichment of *Campylobacter* on Poultry for Enhanced Laboratory System Performance

ABSTRACT

The Food Safety and Inspection Service (FSIS) Field Service Laboratories (FSLs) conducted a two-phase comparison to evaluate the performance of various *Campylobacter* enrichment broths, with the aim of identifying a more efficient enrichment procedure including a reduced incubation period. In the first phase, four enrichment broths, double strength blood-free Bolton's enrichment broth (2x BF-BEB), Hunt broth, *Campylobacter* enrichment broth (Neogen®), and blood-free charcoal (BFC), were assessed for *Campylobacter* recovery for five poultry products. These products included young chicken carcasses, turkey carcasses, chicken parts (legs, breasts, and wings), comminuted chicken and comminuted turkey. 2x BF-BEB and Hunt broth emerged as the most efficient enrichment broth choices, from the combined five product with sensitivities of 94% (95% confidence intervals (CI), 85–98%), and 97% (95% CI, 89.6–99.6), and pooled specificities of 93.8% (95% CI, 84.8–98.3), and 96.8% (95% CI, 89–99.6), respectively. No significant difference in the presence of *Campylobacter* were observed between the two. In the second phase, paired samples were utilized to detect *Campylobacter* in the three chicken product types. Employing a "Gold Standard Approach" across the products, both broths demonstrated equivalent frequencies of positive samples, with moderate agreement in the results for both broths (with highest Kappa value of 0.68 for comminuted chicken). Hunt broth emerged as the optimal replacement for the 2x BF-BEB in the FSIS FSL analytical methods, given its equivalence for *Campylobacter* detection and shorter incubation time.

INTRODUCTION

Campylobacter is a gram-negative, spiral, uniflagellate, microaerophilic bacterium, commonly found in domestic animal populations. *Campylobacter* poses a significant public health threat as the most common bacterial cause of diarrheal illness in the United States (U.S.), with an estimated 1.5 million human illnesses annually in the U.S. alone, according to the Centers for Disease Control (CDC) *(2, 9, 13)*. Most clinically important species of *Campylobacter* have an optimum growth temperature of 41.5

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°C. Birds are attractive hosts for *Campylobacter*, in part due to their higher body temperature of 42°C *(1)*. Raw poultry products exhibit particularly high rates of *Campylobacter* contamination, implicating them in a substantial proportion of campylobacteriosis cases *(4, 10)*. It is therefore important to have optimal detection method for *Campylobacter* in poultry *(9)*.

The Food Safety and Inspection Service (FSIS) Field Service Laboratories (FSLs) monitor *Salmonella* and *Campylobacter* as part of a raw poultry sampling program (Microbiology Laboratory Guidebook (MLG)). The MLG contains test methods used by the FSIS FSLs to support FSIS regulatory activities. FSIS field inspection personnel working in poultry producing establishments around the country collect and send samples to the FSLs for testing. The FSLs report results back to field inspection personnel and establishments once all the tests on a sample are complete and authorized. Initial screening results for *Salmonella* are ready to report in 24 h. However, assessment of samples for the presence of *Campylobacter* required 48 h, delaying sample result reporting.

Campylobacter recovery in the laboratory can be challenging due to its fastidious growing requirements and competitive exclusion. FSIS has continually adapted its *Campylobacter* detection protocols to enhance efficacy and accuracy. These adaptations include transitioning in July 2016 from buffered peptone water (BPW) to neutralizing BPW (nBPW), which counteracts industry-applied microbial intervention carryover *(5)* and, in August 2018, adopting an enrichment method using double strength blood-free Bolton's enrichment broth (2x BF-BEB) at 48 h. Further method revisions in June 2021 involved updating the molecular screening method to the Neogen® Molecular Detection System *(11)*, followed by the adoption of the Bruker MALDI-TOF Biotyper® for final confirmation *(6, 8)*.

This study focuses on identifying and evaluating *Campylobacter* enrichment broths capable of 24-h enrichment time to reduce reporting time for screened negative and confirmed positive samples to enhance laboratory efficiency. Other equally important objectives include maintaining or enhancing technical performance within the FSIS laboratory system and ensuring uniformity across all poultry product types for consistent comparison of laboratory results. The decision-making process for method inclusion involved three phases: market research, preliminary laboratory evaluation and final laboratory evaluation. Criteria such as percent positive results, colony quality, and media selectivity guided the choice of enrichment media for evaluation. Ultimately, two media were chosen for the final laboratory evaluation based on their performance in enriching *Campylobacter* within the desired time frame.

This paper presents the methodology and results of the evaluation process to find an equivalent replacement for the enrichment broth being used, 2x BF-BEB, while shortening the incubation time from 48 h to 24 h and improving time-to-results.

MATERIALS AND METHOD

Study design

This study employed a sequential two-phase comparison approach. The primary objective of the first phase was to compare four enrichment broths for *Campylobacter* recovery: the current 2x BF-BEB incubated for 48 h, and three 24 h broths—Hunt broth, Neogen®, and blood-free charcoal (BFC). Comparisons were conducted across five not ready-to-eat poultry products: young chicken carcasses, turkey carcasses, chicken parts (legs, breasts, and wings), comminuted chicken and comminuted turkey. Samples were inoculated with *Campylobacter* as described below. Following this, 2x BF-BEB and Hunt broth were chosen for use in the second phase.

Phase 2 sought to determine if the broths identified in Phase 1 could replace 2x BF-BEB to detect *Campylobacter* in chicken products using uninoculated samples to assess real-world performance. In the second phase, the most efficient enrichment broth choices, based on differences in *Campylobacter* recovery rates were selected. Paired samples were then used to detect *Campylobacter* in three types of chicken products: chicken parts, young chicken carcasses, and comminuted chicken. A "Gold Standard Approach" (the best available test *(15)*) and "No-gold Standard Approach" *(4)* were employed across these products to determine the suitability of a given broth as a replacement for the 2x BF-BEB in the FSIS FSLs protocol.

Bacterial control strain

The positive control culture for this evaluation was *Campylobacter jejuni* ATCC 33291. This is the accepted culture used by the FSL system for all *Campylobacter* analytical work as stated in MLG 41. Controls for each sample set were plated on trypticase soy agar with 5% sheep blood (SBA) plates and incubated at 42 ± 1°C for 21 ± 3 h, under microaerobic conditions using the Mitsubishi AnaeroPack™ System. The control plate was prepared the day prior to analysis and a fresh control was used for each sample set. The controls for each sample set included a positive culture inoculated media control, an un-inoculated media sterility control and an un-inoculated "blank" matrix control. For the inoculation of positive media controls, colonies were added directly to media using a sterile, non-metal 1 µL loop from the previously inoculated SBA plate.

Broth preparation

Including the 2x BF-BEB reference broth, four enrichment broths were chosen and prepared according to manufactures' instructions and/or as described by the literature.

Double strength blood-free Bolton's enrichment broth (2x BF-BEB)

2x BF-BEB was prepared according to the MLG Appendix 1.04. This formulation was twice as concentrated as the traditional BF-BEB formulation, at a dilution of 1:2 with a 30 ml test portion. The 2x BF-BEB basal ingredients were 20 g meat peptone, 10 g lactalbumin hydrolysate, 10 g yeast extract, 10 g sodium chloride, 1 g sodium pyruvate, 2 g α-ketoglutaric acid, 1 g sodium metabisulfite, 1.2 g sodium carbonate and 0.02 g hemin. Basal ingredients were added to 1 L of distilled water, mixed well and autoclaved at 121°C for 15 min. The broth base was stored cold. Before use, the following antibiotic supplements were added to each liter, 40 mg cefoperazone, 40 mg vancomycin, 40 mg trimethoprim and 20 mg amphotericin B.

***Campylobacter* enrichment broth (Neogen®)**

Neogen® does not require a blood additive nor special equipment for oxygen reduced environments and was prepared as described *(11)*. In a 1.5-liter pre-sterilized screw top container containing a pre-sterilized stir bar, 41 g of powder was suspended in 1,000 ml of sterile purified deionized water. Using the stir bar, the solution was mixed thoroughly then stored cold and protected from light. This broth was used within 24 h of preparation and was brought to room temperature before use, as required by the manufacturer. For this broth, the ratio of enrichment medium to sample was 2:1 to dilute out the interventions, common in samples from establishments, which can interfere with the selective media ingredients.

Blood-free charcoal enrichment broth (BFC)

BFC is also known as charcoal-cefoperazone-polymyxin b-deoxycholate broth *(3)* or blood-free enrichment broth *(14)*. This broth was designed to remove the requirement of blood and special equipment needed for oxygen-reduced environments. BFC was prepared as previously described *(3)*. In a 1.5-L autoclavable screw top container combine: 10 g of beef extract, 10 g of peptone, 5 g of sodium chloride, 3 g of casein hydrolysate, 1 g of sodium deoxycholate, 0.25 g of ferrous sulphate, 0.25 g of sodium pyruvate, 4 g of bacteriological charcoal and 980 ml of distilled water. Apply heat and frequent agitation while mixing to completely dissolve the components. This mixture will result in 1 L of basal broth. Autoclave at 121°C for 15 min. Store the broth base cold and before use add antibiotic supplements to the 1 L: 0.2 g of cefoperazone, 0.2 g of trimethoprim, 0.2 g of vancomycin, 0.1 g of amphotericin B and 100,000 IU/ml of polymyxin b sulfate.

Hunt enrichment broth

In a 1.5-L autoclavable screw top container combine: 10 g of beef extract, 10 g of peptone, 5 g of sodium chloride, 6 g of yeast extract, and 950 ml of distilled water. This mixture resulted in 1 liter of basal broth. Mix well and autoclave at 121°C for 15 min. Store the broth base cold and before use add antibiotic supplements, growth supplements and 50 ml lysed horse blood to the 1 L. Antibiotics to be added: 10 mg of vancomycin-hydrochloride, 12.5 mg of trimethoprim lactate, 15 mg of cefoperazone-sodium, and 20 mg of amphotericin b. Growth supplements to be added: 0.25 g of ferrous sulfate, 0.25 g of sodium metabisulfite, and 0.25 g of sodium pyruvate; or 2 vials per liter of Oxoid™ SRO232E.

Sample matrix types and preparation

All product types were collected as part of FSIS' routine verification sampling of establishments that produce raw poultry products. Collection, sample transportation, and receiving of samples at FSL were done according to previously described standard methods *(7)*.

Chicken carcass rinsates and chicken parts rinsates

Chicken carcass rinsates and chicken parts rinsates were collected and pooled according to sample type from sample reserves that had previously tested "negative" for *Campylobacter*. The pooled rinsate matrices were frozen and thawed five times each to destroy any indigenous *Campylobacter* potentially present. After freezing and thawing they were analyzed following Neogen® Molecular Detection System (MDS) procedures, as described in MLG 41, to confirm they were *Campylobacter* negative. For these rinsate matrices, 30 ± 0.6 ml were then aliquoted into 6" × 9" Whirl-Pak® bags, tightly closed and stored frozen.

Comminuted chicken and comminuted turkey

Comminuted chicken and comminuted turkey products were collected and pooled according to sample type from "negative" sample reserves. These samples were also treated with five freeze-thaws and MDS procedures. Comminuted matrix samples were prepared by aseptically combining 1,625 ± 32.5 ml of BPW and 325 ± 32.5 g of comminuted matrix to a 15" × 20" sterile filtered bag, or equivalent and mixing thoroughly by hand massaging. After mixing, 30 ± 0.6 ml were aliquoted to 6" × 9" Whirl-Pak® bags, tightly closed and stored frozen.

Turkey carcass sponge

Turkey carcass sponge samples do not leave a reserve after enrichment. Turkey carcasses were therefore purchased locally to create samples for the study. Turkey carcasses were sampled as described in FSIS Directive 10,250.1 *(7)*, Sampling Instructions: *Salmonella* and *Campylobacter* Verification Program for Raw Poultry Products. For consistency, analysts used the same supplies that are distributed to the FSIS field inspection personnel that typically collect the samples. First, analysts added 25 ± 0.5 ml of nBPW aseptically to the sponge. Analysts then swabbed the carcass with the moistened sponge using a 5 × 10 cm

template and aseptically replaced the sponge in the sterile bag. Although multiple swabs could be made from a single turkey carcass, no two samples were obtained from the same area on the turkey. These samples were also treated with five freeze-thaws and MDS procedures and stored frozen.

Sample enrichment

The enrichment broths were added to previously prepared samples of each matrix. These samples were not sterile, but were confirmed to be *Campylobacter* negative, as described in the previous section. Each broth required a slightly different sample enrichment method. For 2x BF-BEB, Hunt broth and BFC, 30 ± 0.6 ml were added to the sample bag and for Neogen® 60 ± 0.6 ml were added. For 2x BF-BEB and Hunt broth the sample bags were loosely closed and incubated under microaerobic conditions. BFC and Neogen® were tightly closed with head space removed and were not put under microaerobic conditions. All samples were spiked for fractional recovery and incubated. 2x BF-BEB was incubated for 48 ± 2 h at 42 ± 1°C. All other enrichment broths were incubated for 24 ± 2 h at 42 ± 1°C.

Fractional recovery and inoculation preparation

Fractional recovery is defined as 20–80% positive recovery of the target organism in inoculated samples. To achieve this, a very low concentration of spiking solution is used. Therefore, fractional recovery demonstrates the effectiveness of the method at a low inoculum. The concentration needed is determined experimentally and can vary by sample matrix, enrichment media, growing conditions, and the organism used. Additionally, as each sample is inoculated, it is unknown whether that particular aliquot of spiking solution contains a viable colony forming unit (CFU) of bacteria, creating a blinded experiment with randomly generated, positive samples.

For this broth comparison, the reference broth, 2x BF-BEB, was required to fall in the 20–80% positive range for each set analyzed. If this range was not met, a new sample set was analyzed, possibly with a modified inoculum level to achieve fractional recovery. All *Campylobacter* positive controls were prepared as stated in the Bacterial control strain section above. To create the spiking solution, colonies were suspended in 0.85% saline and adjusted to a McFarland standard of 0.5 (1.5×10^8 CFU/ml). Serial dilutions (10-fold) were done to create a stock suspension that was used to inoculate the matrix at the appropriate concentration to achieve a recovery of 20–80% positive. The spiking solution bacterial counts (CFU/ml) were determined by spreading 1 ml of each countable dilution onto SBA plates and incubating at 42 ± 1°C for 21 ± 3 h, under microaerobic conditions.

Phase 1: Comparison of four enrichment broths for *Campylobacter* recovery in five poultry products

The primary objective of this phase was to compare the current 48 h broth 2x BF-BEB, to 24-h incubations of Neogen®, Hunt broth, and BFC in recovering *Campylobacter* from poultry products.

The lab work was performed by the three FSLs: Eastern Laboratory, Midwestern Laboratory, and Western Laboratory. The analysis was done on a total of 130 samples from the five poultry products including young chicken carcasses, turkey carcasses, chicken parts (legs, breasts, and wings), comminuted chicken and comminuted turkey.

Sample collection for phase 1

A total of 130 samples were randomly collected from five poultry products: comminuted chicken (n = 15), chicken parts rinsates (n = 40), whole chicken rinsates (n = 30), turkey carcass sponges (n = 30) and comminuted turkey (n = 15). These samples were collected at regulated establishments as part of FSIS' routine testing program and represented 520 replicates (130 × 4 medias). Using fractional recovery, each FSL evaluated a minimum of three sample sets per matrix type. Each sample set consisted of four subsets with 10 freeze-thawed matrix samples, a media sterility control, an un-inoculated matrix sterility control and the *Campylobacter* positive matrix control; therefore, each set contained a total of 52 replicates (4 media × (10 samples + 3 controls)). The fractional recovery inoculum was prepared as described above in the Sample matrix types and preparation section; and the amount of inoculum used to spike for fractional recovery varied for each matrix. Samples were spiked, then enriched, analyzed, and confirmed following MLG 41 *(8)*. Only confirmed cultural samples were considered positive for *Campylobacter*. If the current standard 2x BF-BEB sample subset results fell into the fractional recovery range of 20–80% positive, the results for the full set were accepted. Otherwise, the sample set failed and was repeated. This evaluation continued until acceptable results were obtained for three sample sets for each matrix.

Statistical analysis for phase 1

The *Campylobacter* screening was completed using Neogen® Molecular Detection System. The test results (positive/negative) for each sample and medium were compared for cultural confirmation. The percent positive of *Campylobacter* among the four media were compared with Cochran's Q-test and pairwise post-hoc McNemar tests. Performance was evaluated in terms of sensitivity and specificity. Sensitivity represents the probability of a positive test result when the sample is positive, while specificity indicates the probability of a negative test result when the sample is negative. All analyses were performed using R Statistical Software *(12)*.

Phase 2: Comparison of 2x BF-BEB and selected broth using paired samples to detect *Campylobacter* in three chicken product types

Phase 2 aimed to determine if the broths identified in Phase 1 were an appropriate replacement for 2x BF-BEB in the FSIS FSL protocol to detect *Campylobacter* in chicken products.

Sample collection for phase 2

FSLs compared the new MLG method to the previous method (2x BF-BEB) using uninoculated sample reserves to assess real-world performance without the influence of freeze-thaw cycles on the background flora. Analysts conducted a side-by-side study on 200 chicken parts rinsates, 200 whole chicken rinsates and 400 comminuted chicken samples over a four- month period. During sample enrichment with 2x BF-BEB, FSLs set aside whole chicken rinsates with excess reserve for the study. Analysts removed 30 ml aliquots from the reserve. Comminuted chicken regulatory samples with reserves of at least 325 g were also selected for the study. The study samples were enriched with Hunt broth. Analysts incubated the 2x BF-BEB for 48 ± 2 h and Hunt broth samples for 24 ± 2 h. All samples were confirmed per the MLG Chapter 41 method. Results for both media were compared to the cultural results using a "Gold Standard Approach" across the products.

Statistical analysis for phase 2

A range of statistical methods was employed to compare the performance of lab techniques. McNemar's Chi-square statistic and Kappa statistic were used to assess agreement between the broths at the screening phase. Sensitivity and specificity for each broth at the screening stage were estimated using a "Gold Standard Approach." Additionally, a "No-gold Standard Approach" was utilized to estimate latent sensitivity and specificity parameters. The Bayesian model was based on 5 million iterations with resampling of 100,000 (i.e., N = 5,000,000 and m = 100,000) as described previously *(4)*. The effect of changing the enrichment broth from 2x BF-BEB to an alternative was examined for chicken carcasses, parts, and comminuted products.

For each broth-product combination, we examine the proportion of screen-positive samples that were found positive following confirmatory culture. Nevertheless, paired broth samples come from the same original rinse (carcasses or parts) or comminuted product sample; therefore, the true contamination status of the original sample is better informed by considering the confirmatory test results from both broths. We can define a "Gold Standard Approach" designation by assuming any sample confirmed as positive for either broth is contaminated and any sample that is confirmed negative for both broths is not contaminated; we partitioned the samples according to this in a two-by-two classification scheme. This "Gold Standard Approach" allows direct estimation of sensitivity and specificity for each broth at the screening phase.

Finally, we can use a "No-gold Standard Approach" to estimate the latent sensitivity and specificity parameters that best explain the observed paired screening results. Nevertheless, this method is not readily adaptable to inclusion of the confirmatory results because screen-negative samples were not subjected to culturing.

To examine the effect of changing the enrichment broth from 2x BF-BEB, we begin by assuming that the specificity of identifying *Campylobacter*-positive samples is 100%, regardless of broth. Because any positive sample, regardless of broth used during screening, is subjected to a culture assay, an assumption of perfect specificity implies there are no samples that have a false-positive result after culture. All analyses were performed using R Statistical Software *(12)*.

RESULTS

Results for Phase 1: Comparison of four enrichment broths for *Campylobacter* recovery in five poultry products

Table 1 shows the *Campylobacter* percent positive comparison between 2x BF-BEB, Hunt broth, Neogen® and BFC for all poultry products combined. The percent positive of *Campylobacter* using Hunt broth (52%) and 2x BF-BEB (51%) were higher and significantly different compared to Neogen® and BFC using the Cochran's Q test and pairwise post-hoc McNemar test.

The *Campylobacter* percent positive using 2X BF-BEB and Hunt broth was relatively high across all product types. The *Campylobacter* percent positive using Neogen® (6.7 – 47%) and BFC (0 – 53%) was low. Across all products and diagnostic test media, the highest percent positive was for turkey carcass sponges (87%) using Hunt broth and the lowest was for turkey carcass sponges (0%) using BFC.

Comparison of the overall sample test results in 2x BF-BEB, Hunt, Neogen® and BFC for combined product

The result for each of the four diagnostic test media was compared to the corresponding test result of cultural confirmation test (media/broth) for each media. When the five product type results are combined, the percent positive for each of the media types and cultural confirmation is similar, except for Neogen®, as shown in the last row of *Table 1*.

For the combined product, the percentage of positive *Campylobacter* samples using 2x BF-BEB and Hunt broth are consistently higher, as compared to the other medias. This improved detection limit was also shown using cultural confirmation, suggesting a low false positive and false negative rate.

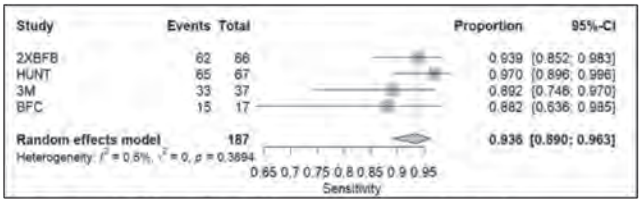
Comparison of sensitivity and specificity 2x BF-BEB, Hunt, Neogen® and BFC by combined product type

To further compare the performance of the media, we calculated the expected values and 95% bounds of the sensitivity and specificity. We used the Cochran-Q test to assess heterogeneity of sensitivities and specificities, the null hypothesis being that for each product sample in each enrichment method, all the sensitivities (specificities) are equal.

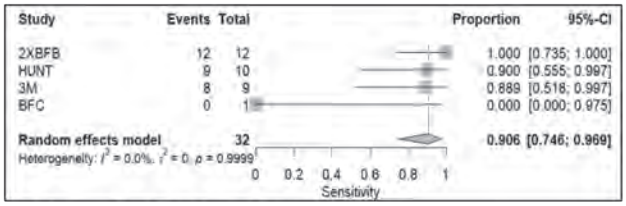
TABLE 1. The number of *Campylobacter* positive samples and percent positive for each of the four diagnostic test media and for the five poultry products comparison using the Cochran's Q-test and pairwise post-hoc McNemar tests

Product (N)	2x BF-BEB n (%) [a]	Hunt n (%) [a]	Neogen® n (%) [b]	BFC n (%) [c]
Comminuted Chicken (N=15)	10 (67)	5 (33)	7 (47)	7 (47)
Chicken Parts Rinsates (N = 40)	23 (57)	15 (38)	12 (30)	1 (2.5)
Turkey Carcass Sponges (N = 30)	14 (47)	26 (87)	2 (6.7)	0 (0)
Whole Chicken Rinsates (N = 30)	12 (40)	10 (33)	9 (30)	1 (3.3)
Comminuted Turkey (N = 15)	7 (47)	11 (73)	7 (47)	8 (53)
Total (N = 130)	66 (51)	67 (52)	37 (28)	17 (13)
Confirmation n (%)	66 (51)	67 (52)	47 (36)	17 (12)

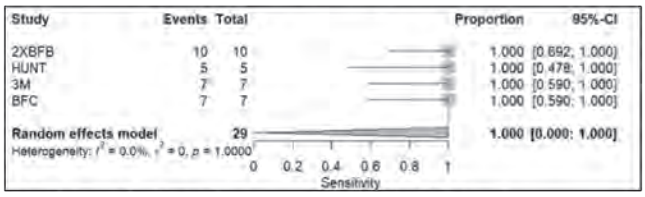
N = total number of samples. n = number of positive *Campylobacter* samples. Double strength blood-free Bolton's enrichment broth (2x BF-BEB), Hunt broth (Hunt), *Campylobacter* enrichment broth (Neogen®), and blood-free charcoal (BFC). Different letters (a, b, c) indicate statistically significant differences ($P<0.05$).



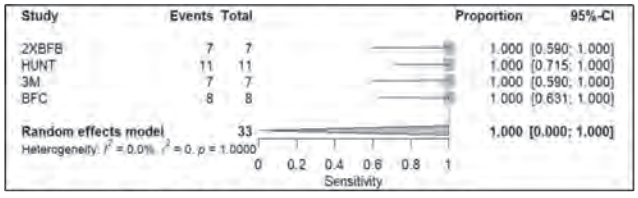
A1: Sensitivity for all products combined



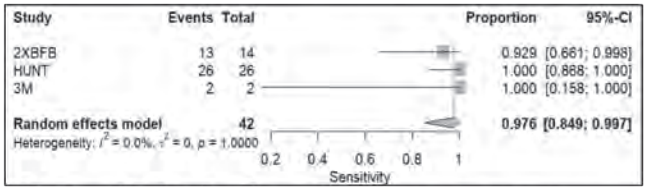
B1: Sensitivity for whole chicken



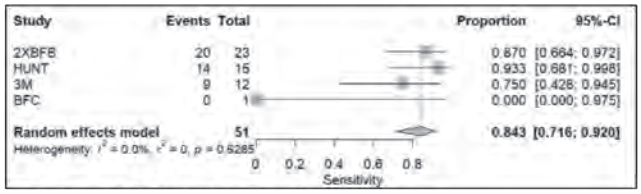
C1: Sensitivity for comminuted chicken



D1: Sensitivity for comminuted turkey



E1: Sensitivity for the turkey carcass sponges



F1: Sensitivity for the chicken parts

Figure 1. Comparative paired forest plot of sensitivity of *Campylobacter* test results of the four enrichment broths, double strength blood-free Bolton's enrichment broth (2x BF-BEB), Hunt broth, *Campylobacter* enrichment broth (Neogen®), and blood-free charcoal (BFC) for the five selected poultry products.

The results of the analysis for combined product sensitivity and specificity with their 95% confidence intervals are shown in *Figure 1 A1* and *Figure 2 A2*. The sensitivity and specificity of Hunt and 2x BF-BEB are both above 90%. The hypothesis test for equality of sensitivities across all media is accepted (test for equality of sensitivities: I^2 = 0.5% [0.0%; 84.8%], df = 3, *P*-value = 0.344), and hence there is no significant difference in sensitivity by media. However, the test for equality of specificities is rejected (I^2 = 0.955 [0.874; 0.985], df = 3, *P*-value = 0.0054), and hence there is a significant difference in specificity. As shown in *Figure 1 A1* & *Figure 2 A2*, the overall proportion of the random effect model for the sensitivity and specificity were 0.936 (95% CI 0.890 – 0.985) and 0.955 (95% CI 0.874 – 0.985), respectively. The Hunt broth has the highest sensitivity and specificity suggesting it is the best media for *Campylobacter* detection among the combined product sample, followed by 2x BF-BEB.

Quantifying heterogeneity of sensitivities and specificities for 2x BF-BEB, Hunt, Neogen® and BFC by individual product type

Whole chicken rinsates

The result of the diagnostic tests for whole chicken rinsates (n=30) for each of the four enrichment technologies is shown in *Figure 1 B1* and *Figure 2 B2*. The hypothesis test for equality of both sensitivities and specificities across all media is accepted. The heterogeneity, defined as the variation in the sensitivity and specificity between the media, was not significant (I2 is 0% [0.0%; 84.7%]; and Cochran-Q >0.05), the random-effect model was used in the current analysis. Thus, both the sensitivities and specificities among the medias are not significantly different. As shown in *Figure 1 B1* and *Figure 2 B2*, the overall sensitivity and specificity for differentiating the positive from the negative were 0.906 (95% CI 0.746 – 0.969) and 0.994 (95% CI 0.474 – 1), respectively. The wide confidence bounds for the paired forest plots indicated the sensitivity of BFC and specificity of Neogen® was highly variable in whole chicken rinsates.

Comminuted chicken

The result of the diagnostic tests for comminuted chicken (n=15) for each of the four enrichment broths is shown in *Figure 1 C1* and *Figure 2 C2*. The hypothesis test for equality of both sensitivities and specificities across all media was accepted (I^2 is 0% [0.0%; 84.7%]; and Cochran-Q = 0.9999). Thus, there were no significant differences in sensitivities and specificities among the media. However, visual evaluation of the paired forest plots *Figure 1 C1* and *Figure 2 C2* – indicated high variability in sensitivity and specificity across all media, except for the specificity of Neogen®.

Comminuted turkey

The result of the diagnostic tests for comminuted turkey (n=15) for each of the four enrichment broths is shown in *Figure 1 D1* and *Figure 2 D2*. The hypothesis test for equality of both sensitivities and specificities across all media is accepted. The heterogeneity between the media was not significant (I^2 is 0% [0.0%; 84.7%]; and Cochran-Q = 0.9999). Thus, both the sensitivities and specificities among the medias are not significantly different, as shown in *Figure 1 D1* and *Figure 2 D2*. Given the expected value of 100%, the analysis of the variability by visual evaluation of the paired forest plots indicated very high variability for sensitivity and specificity across all the media, except for the specificity of BFC (85%).

Turkey carcass sponges

The result of the diagnostic tests for turkey carcass sponges (n=30) for each of the four enrichment broths is shown in *Figure 1 E1* and *Figure 2 E2*. The hypothesis test for equality of both sensitivities and specificities across all media is accepted. The heterogeneity between the media was not significant (I^2 is 0% [0.0%; 84.7%]. The Cochran-Q values are 0.6285 and 0.4210 for sensitivity and specificity respectively. Thus, both the sensitivities and specificities among the medias are not significantly different. The analysis of the variability by visual evaluation of the paired forest plots indicated moderate variability for sensitivity and specificity across all the media, except for specificity of BFC (0% [0%;98%]).

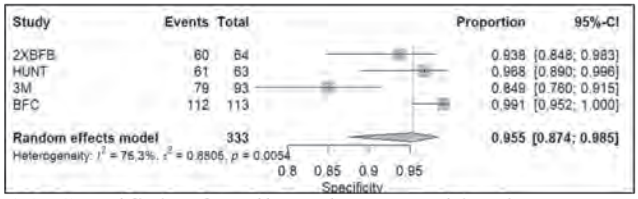
Chicken parts rinsates

The result of the diagnostic tests for chicken parts rinsates (n=40) for each of the four enrichment broths is shown in *Figure 1 F1* and *Figure 2 F2*. The hypothesis test for equality of both sensitivities and specificities across all the vendors is accepted. The heterogeneity between the media was not significant (I^2 is 0% [0.0%; 84.7%]. The Cochran-Q values are 0.6285 and 0.4210 for sensitivity and specificity, respectively. Thus, both the sensitivities and specificities among the medias are not significantly different. The analysis of the variability by visual evaluation of the paired forest plots indicated moderate variability for sensitivity and specificity across all the media, except for specificity of BFC (0% [0%;98%]).

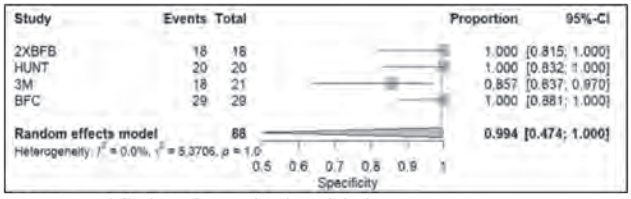
Results for phase 2: "Gold -Standard Approach" whole chicken rinsates

The Hunt broth detected 28.5% (57/200) positive *Campylobacter* samples and the 2x BF-BEB detected 24% (48/200) positive samples *(Table 2 A)*. The McNemar's test does not reject the hypothesis that both broths perform the same. The Kappa statistic suggests moderate agreement between the results for both broths.

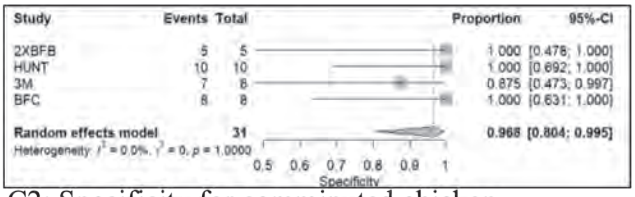
The "Gold Standard Approach" analysis suggests the Hunt broth has a sensitivity of 82% and the 2x BF-BEB



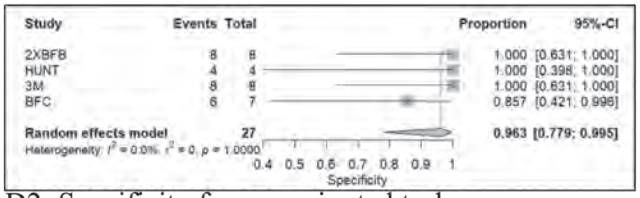
A2: Specificity for all products combined



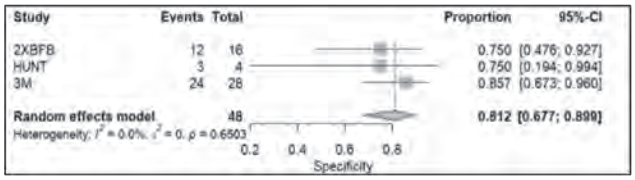
B2: Specificity for whole chicken



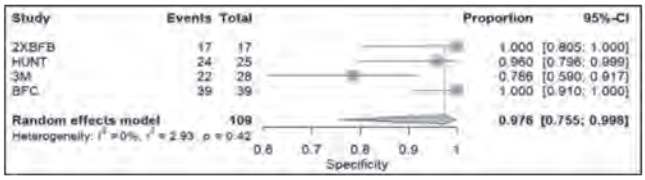
C2: Specificity for comminuted chicken



D2: Specificity for comminuted turkey



E2: Specificity for turkey carcass sponges



F2: Specificity for chicken parts

Figure 2. Comparative paired forest plot of specificity of *Campylobacter* test results of the four enrichment broths, double strength blood-free Bolton's enrichment broth (2x BF-BEB), Hunt broth, *Campylobacter* enrichment broth (Neogen®), and blood-free charcoal (BFC) for the five selected poultry products.

TABLE 2. *Campylobacter* test results comparisons of Hunt broth and 2x BF-BEB in paired samples across the three products A) whole chicken rinsates, B) chicken parts rinsates, and C) comminuted chicken

A) Whole Chicken Rinsates				
	2x			
H		POS	NEG	Total
	POS	31	26	57
	NEG	17	126	143
	Total	48	152	200
McNamar P=0.13, Kappa = 0.45				

B) Chicken Parts Rinsates				
	2x			
H		POS	NEG	Total
	POS	26	21	47
	NEG	14	139	153
	Total	40	160	200
McNamar P=0.18, Kappa = 0.49				

C) Comminuted Chicken				
	2x			
H		POS	NEG	Total
	POS	31	10	41
	NEG	15	338	353
	Total	46	348	394
McNamar P=0.42, Kappa = 0.68				

H, Hunt broth; 2x, 2x BF-BEB broth; NEG, test results negative; POS, test results positive

TABLE 3. "Gold Standard Approach" analysis of paired *Campylobacter* test results using either Hunt broth or 2x BF-BEB across the three products A) whole chicken rinsates; B) chicken parts rinsates; C) comminuted chicken

A) Confirmation: Whole Chicken Rinsates									
		POS H or 2x	NEG H and 2x				POS H or 2x	NEG H and 2x	Total
H	POS	42	13	55	2x	POS	38	9	47
	NEG	9	129	138		NEG	13	133	146
	Total	51	142	193		Total	51	142	193
H Se - 82%; H Sp = 91%					*2x Se = 75% ; 2x Sp = 94%*				

B) Confirmation: Chicken Parts Rinsates									
		POS H or 2x	NEG H and 2x				POS H or 2x	NEG H and 2x	Total
H	POS	39	8	47	2x	POS	38	2	40
	NEG	13	140	153		NEG	14	146	160
	Total	52	148	200		Total	52	148	200
H Se - 75%; H Sp = 95%					*2x Se = 73% ; 2x Sp = 99%*				

C) Confirmation: Comminuted Chicken									
		POS H or 2x	NEG H and 2x				POS H or 2x	NEG H and 2x	Total
H	POS	39	8	47	2x	POS	38	2	40
	NEG	13	140	153		NEG	14	146	160
	Total	52	148	200		Total	52	148	200
H Se - 77%; H Sp = 98%					*2x Se = 91% ; 2x Sp = 98%*				

H, Hunt broth; 2x, 2x BF-BEB broth; NEG, test results negative; POS, test results positive; Se, Sensitivity; Sp, Specificity.

has a sensitivity of 75%; the specificities implied using this analysis are both > 90% *(Table 3 A)*. A sample is assumed contaminated if it was confirmed positive by either the Hunt broth or 2x BF-BEB; it is assumed non-contaminated if it was confirmed negative by both broths.

"Gold Standard Approach" chicken parts rinsates

The Hunt broth detected 23.5% (47/200) of positive samples and the 2x BF-BEB detected 20% (40/200) of positive samples *(Table 2 B)*. The McNemar's test does not reject the hypothesis that both broths perform the same. The Kappa statistic suggests moderate agreement between the results for both broths. The "Gold Standard" analysis suggests the Hunt broth has a sensitivity of 75% and the 2x BF-BEB has a sensitivity of 73%; the specificities implied using this analysis are both >90% *(Table 3 B)*.

"Gold Standard Approach" comminuted chicken sampling

The Hunt broth detected 10.4% (41/394) of positive samples and the 2x BF-BEB detected 11.7% (46/394) of positive samples *(Table 2 C)*. The McNemar's test does not reject the hypothesis that both broths perform the same. The Kappa statistic suggests moderate-strong agreement between the results for both broths.

The "Gold Standard" analysis suggests the Hunt broth has a sensitivity of 77% and the 2x BF-BEB has a sensitivity of 91%; the specificities implied using this analysis are both >90% *(Table 3 C)*.

TABLE 4. "No-gold Standard Approach" analysis of rapid screen results for the whole chicken rinsates, chicken parts rinsates, and comminuted chicken

	Whole mean (95% CI)	Parts mean (95% CI)	Comminuted mean (95% CI)
Hunt Sensitivity	86% (62 - 99)	87% (62 - 99)	88% (68 - 99)
Hunt Specificity	84% (75 - 97)	87% (79 - 99)	95% (90 - 99)
2X BF-BEB Sensitivity	87% (58 - 99)	87% (59 - 99)	88% (68 - 99)
2X BF-BEB Specificity	89% (80 - 99)	91% (62 - 99)	92% (84 - 99)

CI = confidence intervals

"No-gold Standard Approach"

The "No-gold Standard Approach" finds little difference in the estimated sensitivities of the 2x BF-BEB and Hunt broth across the three product types *(Table 4)*. This approach suggests that the specificity of 2x BF-BEB is somewhat higher than the Hunt broth for whole chicken rinsates and chicken parts rinsates, but this relationship is reversed for comminuted chicken.

CONCLUSIONS

The result of Phase 1 analysis revealed that among the four broths evaluated, when all five poultry products were combined, 2x BF-BEB and Hunt broth emerged as the most efficient choices for recovery of *Campylobacter*. There was no significant difference in the percentage of *Campylobacter* observed between the two broths. Phase 2 was used to determine which one of these broths would be most efficient and best suitable for the FSIS FSL protocol. Both broths exhibited high accuracy in detecting *Campylobacter* across the three chicken products. The "Gold Standard Approach" and the "No-gold Standard Approach" analysis showed comparable sensitivity and specificity, with no significant difference in performance, despite a shorter 24-h enrichment time for the Hunt broth. Incorporation of the Hunt broth for enrichment ensures consistent comparison of laboratory results across different poultry products, decreases time to report results, and improves uniformity by using a single media for all product types considered. Overall, the analysis concludes that Hunt broth at 24 h is a suitable replacement for 2x BF-BEB at 48 h in the FSIS FSL protocol. Evaluating different *Campylobacter* enrichment medias is an example of FSIS' commitment to continually modernize methods and equipment.

ACKNOWLEDGMENTS

The authors thank the FSIS Eastern Field Service Laboratory (Microbiology Screening and Microbiology Characterization Branches) and the Microbiology sections of the FSIS Midwestern and Western Field Service laboratories for the analyses conducted and data collected. The authors also thank Dr. Eric Ebel for his work on the "No-gold Standard Approach".

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