Saturday

256

Comparative Evaluation of Puritan® and Copan Cary Blair Medium for Recovery of Enteric Pathogens Using Automated Plating

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Revised Abstract

Background: Cary Blair medium (CB) is commonly used for preservation of fecal specimens. CB is typically manually plated to selective and differential media for culture of enteric pathogens. Tubes with modified CB are now available from Puritan Medical Products (P) and Copan Diagnostics (C) for use with automated plating systems.

Methods: ATCC and clinical strains of *Salmonella* (SA), *Shigella* (SH), *Y. enterocolitica* (YE), *E.coli* O157 (ECO157), *C. jejuni* (CJ) and *V. parahaemolyticus* (VP) were tested alone and with a mixture of *E. coli* (EC) and *E. faecalis* (EF) to simulate normal fecal flora (EC/EF). CBs were inoculated with 100 uL of approx. 10^7 cfu/mL. At 0 h, CBs were loaded onto a BD Kiestra InoqulA programmed to dispense 30 uL onto SBA, MAC, XLD, CIN, MACS, and CSM plates with magnetic bead 4 quadrant streaking. One set of CBs was maintained at 22-25ºC (RT) with plating at 24 and 48 h. A second set of CBs was maintained at 2-8ºC (RFT) with plating at 24, 48 and 72 h. Growth was graded as 1+, 2+, 3+ and 4+ at each time point. A change of 2 or more grades as compared to time 0 h was considered significant.

Results: When CBs were maintained at RT the quantity of growth of SA, SH, YE and EC0157 alone did not change significantly at 48 h compared to 0 h for P but did change for some SA in C. When tested with EC/EF, there were significant decreases in some SA and SH most notably in C. For CJ, at 24 h there was no significant change in quantity of growth for either P or C, however, for some strains there were significant decreases in growth at 48 h with both P and C. For VP tested alone or with EC/EF, growth from P was significantly lower at 48 h. When CBs were maintained at RFT, growth of SH alone at 72 h did not change significantly for both P and C, however, at 72 h significant decreases were seen with some strains of SA alone and some strains of SH and SA with EC/EF most notably in C. For some strains of ECO157 alone or with EC/EF significant changes were seen at 72 h in both P and C. For YE and CJ alone or with EC/EF there were no significant changes in growth at 72 h for both P and C. For VP at 72 h the quantity of growth was significantly decreased for P when tested alone or with EC/EF.

Conclusions: P and C tubes containing modified CB allowed for streamlined, consistent and quality streaking on an automated plating platform. In our study, P performed better than C at maintaining viability of SA and SH for 48 h at RT and 72 h at RFT in simulated fecal samples. P and C were comparable at maintaining viability at RT and RFT for YE, ECO157 and CJ. For VP, C performed better than P at RT and RFT. For optimum recovery of enteric pathogens using P or C, storage should be limited to 24 hours at RT and 48 at RFT.

Introduction

The microbiology laboratory plays a key role in the diagnosis of diarrheal illnesses. Enteric infections can be caused by any one of several different bacterial pathogens. Stool culture procedures are typically designed to screen for the most common pathogens: *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. Many labs also routinely screen for *E. coli* O157:H7 and *Yersinia* spp. Also, *Vibrio* spp. culture may on occasion be requested if there is a high suspicion for this pathogen. The first step in the diagnosis of enteric infections involves the collection and preservation of stool samples during transport to the laboratory. One of the most common transport media designed to maintain the viability of the enteric pathogens described above is modified Cary Blair medium (CB). This medium is a nonnutritive balanced salt solution containing phosphate buffer to maintain an appropriately high pH. Chloride salts to provide essential ions that help maintain osmotic balance. Agar gives a semisolid texture to the medium. Sodium salts and L-cysteine provide a reduced environment. Once received in the laboratory, CB is typically manually plated to selective and differential media for culture of enteric pathogens. Tubes with CB are now available from Puritan Medical Products – Puritan® Cary Blair Medium (P) and Copan Diagnostics – FecalSwab™ medium (C) for use with automated plating systems.

Methods

Bacterial strains: *Salmonella typhimurium* ATCC 14028, *Shigella flexneri* ATCC 12022, *Yersinia enterocolitica* (YE) ATCC 9610, *E.coli* O157 (ECO157) ATCC 700728, *Campylobacter jejuni* (CJ) ATCC 33291 and *Vibrio parahaemolyticus* (VP) ATCC 17802 as well as clinical isolates of *Salmonella* spp. (SA), *Shigella* spp. (SH), YE, ECO157, and CJ were tested alone and with a mixture of *E. coli* ATCC 25922 (EC) and *E. faecalis* ATCC 29212 (EF) to simulate normal fecal flora (EC/EF). Enteric pathogen and EC and EF isolates were suspended in sterile demineralized water to a turbidity equivalent to a 0.5 McFarland standard. For enteric pathogens tested alone, a tenfold dilution of the suspension was performed in sterile demineralized water. For combination studies, equal volumes of the enteric pathogen and EC and EF suspensions were combined and then diluted tenfold.

Methods continued

Stability study: For each test, 100 uL of the diluted suspensions were placed into the CBs. At 0 hr, all CBs were loaded onto a BD Kiestra™ InoqulA™ programmed for fully automated mode to dispense 30 uL onto trypticase soy agar with 5% sheep blood, MacConkey agar, XLD agar, CIN agar, MacConkey Sorbitol agar, and Campylobacter Selective Medium agar plates. Four quadrant streaking was performed by the automated system. One set of CBs was maintained at room temperature (22-25ºC) (RT) with plating at 24 and 48 hrs. A second set of CBs was maintained at refrigerator temperature (2-8ºC) (RFT) with plating at 24, 48 and 72 hrs. Growth was graded as 1+, 2+, 3+ and 4+ at each time point. A decrease of 2 or more grades as compared to time 0 hr was considered significant.

Table 1. Enteric pathogen stability in modified Cary Blair at room temperature (22-25ºC)[a]

		Number with stable colony count (%)			
		Alone		With EC/EF	
		24 hr	48 hr	24 hr	48 hr
Salmonella n=15	Puritan	15 (100)	15 (100)	15 (100)	15 (100)
	Copan	13 (87)	12 (80)	14 (93)	13 (87)
Shigella n=7	Puritan	7 (100)	7 (100)	7 (100)	6 (86)
	Copan	7 (100)	7 (100)	6 (86)	6 (86)
E.coli O157:H7 n=5	Puritan	5 (100)	5 (100)	5 (100)	5 (100)
	Copan	5 (100)	5 (100)	4 (80)	4 (80)
Campylobacter n=5	Puritan	5 (100)	2 (40)	5 (100)	2 (40)
	Copan	5 (100)	3 (60)	5 (100)	3 (60)
Yersinia n=3	Puritan	3 (100)	3 (100)	3 (100)	3 (100)
	Copan	3 (100)	3 (100)	3 (100)	2 (67)
Vibrio n=1	Puritan	0 (0)	0 (0)	0 (0)	0 (0)
	Copan	1 (100)	1 (100)	1 (100)	1 (100)

[a] Stable colony count defined as no decrease or less than 2 grade decrease as compared to time 0 hour

Table 2. Enteric pathogen stability in modified Cary Blair at refrigerator temperature (2-8ºC)[a]

		Number with stable colony count (%)					
		Alone			With EC/EF		
		24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
Salmonella n=15	Puritan	15 (100)	14 (93)	13 (87)	15 (100)	15 (100)	13 (87)
	Copan	12 (80)	10 (67)	8 (53)	15 (100)	15 (100)	9 (60)
Shigella n=7	Puritan	7 (100)	7 (100)	7 (100)	7 (100)	7 (100)	6 (86)
	Copan	7 (100)	7 (100)	7 (100)	7 (100)	7 (100)	6 (86)
E.coli O157:H7 n=5	Puritan	4 (80)	4 (80)	4 (80)	4 (80)	4 (80)	4 (80)
	Copan	4 (80)	4 (80)	4 (80)	3 (60)	3 (60)	3 (60)
Yersinia n=3	Puritan	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)
	Copan	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)
Campylobacter n=1	Puritan	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
	Copan	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Vibrio n=1	Puritan	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Copan	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)

[a] Stable colony count defined as no decrease or less than 2 grade decrease as compared to time 0 hour.

Results

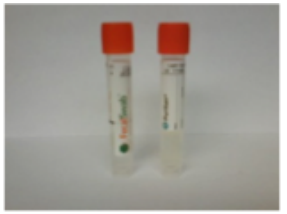
When CBs were maintained at RT the quantity of growth of SA alone or with EC/EF did not change significantly at 48 hrs compared to 0 hr for P, however, significant reductions in colony counts for some strains occurred as early as 24 hrs for C CB. For SH alone, colony counts remained stable in both P and C for 48 hrs, but when tested with EC/EF, there were significant decreases for one strain in both P and C at 48 hrs and as early as 24 hrs in C CB. YE and EC0157 alone did not change significantly at 48 hrs compared to 0 hr for P and C, however, when mixed with EC/EF there were significant changes starting at 24 hrs for one strain of ECO157 and at 48 hrs for one strain of YE in C. For CJ, at 24 hrs there was no significant change in quantity of growth for either P or C, however, for some strains, tested alone or with EC/EF, there were significant decreases in growth at 48 hrs with both P and C CB. For VP tested alone or with EC/EF, growth from P was significantly lower than C starting at 24 hrs (Table 1).

When CBs were maintained at refrigerated temperature, growth of SA alone and with EC/EF was reduced for some strains in P and C with some changes occurring as early as 24 hrs with C CB. Growth of SH alone remained stable for 72 hrs in P and C, but when with EC/EF there were significant decreases for one strain in both P and C CB. For ECO157 alone or with EC/EF significant changes were seen starting at 24 hrs in both P and C CB. For YE and CJ alone or with EC/EF there were no significant changes in growth at 72 h for both P and C CB. For VP starting at 48 hrs the quantity of growth was significantly decreased for P when tested alone or with EC/EF (Table 2). No specimen related mechanical errors occurred with the fully automated plating system used in this study.

Conclusions

Fecal samples collected for culture and placed into an appropriate preservative such as modified Cary Blair medium should ideally be transported to the laboratory within 2 hours of collection. In many healthcare systems, the majority of these specimens are collected a significant distance away from the laboratory and transport times of greater than 2 hours are common. The package insert accompanying C CB indicates that if specimen processing is delayed, then specimens should be refrigerated at 2-8ºC and processed within 72 hours or maintained at room temperature (22-25ºC) and processed within 48 hours. The package insert for P CB makes no recommendations for storage temperatures or time limits but indicates that best results are achieved when specimens are processed shortly after the time of collection. In our study we found that P performed better than C at maintaining viability of SA and SH for a longer time at RT and RFT in simulated fecal samples. P and C were comparable at maintaining viability at RT and RFT for YE, ECO157 and CJ. For VP, C performed better than P at RT and RFT. Decreased colony counts of enteric pathogens in preserved specimens due to processing delays reduces the likelihood of enteric pathogen recovery by stool culture. For optimum recovery of enteric pathogens using P or C, storage should be limited to 24 hrs at RT and 48 hrs at RFT.

Both P and C CB tubes containing modified Cary Blair allowed for streamlined specimen handling and consistent and quality streaking on an automated plating platform with no mechanical sampling errors.



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