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Microbial recovery from clot-activating Vacutainers®

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ABSTRACT

Biological specimens for microbiological analysis are often collected in BD Vacutainers®, which are not specifically designed for microbial recovery. Bacterial and fungal recovery was analyzed for glass and plastic tubes with or without clot-activating silica. No significant impact was found for the recovery of most bacteria and yeasts tested, however, *Haemophilus influenzae* recovery from cerebrospinal fluid was significantly reduced in both glass and plastic clot activator tubes.

1. Introduction

Clinical microbiology laboratories may receive normally sterile body fluids for culture in sterile "red top" BD Vacutainers®, which are FDA approved blood and serum collection tubes for chemistry and immunohematology analysis, not bacterial or fungal recovery. While BD recommends clot activator-containing Vacutainer® (CAV) tubes for use in downstream infectious disease diagnostics, they note the performance characteristics require validation prior to use; a detail that some labs may overlook given that other FDA-approved microbiology transport systems like the ESwab™ do not require verification prior to use (Miller et al., 2013). CAVs are coated with silicone and contain clot activating silica beads that are, according to a patent, "glass particle(s) having a surface area of the native, unmodified glass which activates the intrinsic coagulation pathway. A second surface area of the particle has an activator of the extrinsic pathway immobilized thereon" (Vogler & Graper, 1995). The stability and viability of organisms exposed to these beads is not known, but should be studied given that otherwise innocuous materials like wood, cotton, and anticoagulants can have detrimental effects on recovery of some microorganisms (Jorgensen & Pfaller, 2015).

The purpose of this study was to analyze recovery of clinically relevant gram positive bacteria, gram negative bacteria and yeast incubated in CAVs. The first step was to determine the specimen sources that are most frequently submitted in CAVs. A retrospective review of SOP (Standard Operating Procedure) exception requests revealed that 45 specimens were submitted for culture in glass or plastic CAVs within the 1.5 year period between January 2013 and June 2014 at the studied tertiary care center. Specimen sources included 26 (58%) joint fluids, 9 (20%) peritoneal fluids, 4 (9%) tissue sources, 3 (7%) vaginal sources, 2 (4%) pleural fluids, and 1 (2%) cerebrospinal fluid (CSF). Overall, less than 1% of each specimen-type received for culture was collected in CAVs, except joint fluids (3.3% of 775 specimens) and peritoneal fluids (1.5% of 576 specimens). Bacterial and fungal species commonly isolated from these specimen types were then retrospectively compiled from the same time period (Table 1).

2. Materials and methods

12 clinical isolates representing the most relevant and commonly isolated bacterial and fungal species from joint and peritoneal fluids, and laboratory QC strains of *Streptococcus pneumonia* (ATCC 49619), *Haemophilus influenza* (ATCC 49247) and *Cryptococcus neoformans* (ATCC 32045) were pulled from frozen stock cultures and cultivated for two generations on blood agar (BD BBL, Sparks, MD) or chocolate agar (BD BBL) for bacteria at 35 °C and Sabouraud brain heart infusion agar (BD BBL) for yeasts at 30 °C. Isolates were then diluted in remnant culture negative body fluids including peritoneal fluid, joint fluid or CSF, as indicated in Table 2, to roughly 1×10^3 colony forming units per milliliter (cfu/ml) bacteria and 2×10^3 cfu/ml yeast based on the assumption that a 0.5 MacFarland is equal to 1.5×10^8 cfu/ml of bacteria (Clinical and Laboratory Standards Institute (CLSI), 2009a) or 5×10^6 cfu/ml of yeast (Clinical and Laboratory Standards Institute (CLSI), 2009b). Two fastidious isolates, *S. pneumoniae and H. influenzae*, due to much lower recovery rate after seeding, were diluted to roughly 1×10^4 cfu/ml (less dilute compared with other isolates to account for decreased viability in the tested conditions) in CSF. 500 μL of each suspension was then pipetted in triplicate to each of the following Vacutainer types available to physicians at this institution: plastic 6 mL red top BD Vacutainers® containing clot activating silica beads (PCA), glass 10 mL red top BD Vacutainers® containing clot activating silica beads (GCA), and plastic 6 mL clear top BD Vacutainers® containing no additives (PNA). All tubes were inverted for five times and then incubated for 18 hours at room temperature to simulate typical

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Table 1
Most commonly isolated organisms from joint and peritoneal fluids. All positive cultures submitted during an 18 month period were included for analysis, regardless of transport tube.

	Joint fluid isolates (n = 100)	Peritoneal fluid isolates (n = 210)	CSF isolates (n = 32)
Staphylococcus aureus	28%	-	3%
Beta hemolytic *Streptococcus* species	9%	-	3%
Streptococcus pneumoniae	-	-	3%
Enterococcus species	7%	23%	-
Coagulase negative *Staphylococcus*	31%	10%	38%
Escherichia coli	-	14%	-
Enterobacter cloacae complex	3%	2%	3%
Candida albicans	-	15%	-
Candida glabrata	-	10%	-
Cryptococcus neoformans	-	-	16%
Anaerobic organisms	12%	11%	16%
Other bacterial or fungal species	10%	13%	16%

Table 2
Isolate and test information.

	Category	Source of isolates	Dilution matrix			# isolates tested
			Joint fluid	Peritoneal fluid	CSF	
Escherichia coli	GN	Clinical	✓	✓	✓	2
Enterobacter cloacae	GN	Clinical	✓	✓	-	1
Enterococcus faecium	GP	Clinical	-	✓	-	2
Enterococcus faecalis	GP	Clinical	✓	-	-	1
Staphylococcus aureus	GP	Clinical	✓	✓	-	2
Staphylococcus epidermidis	GP	Clinical	✓	-	✓	1
Streptococcus agalactiae	GP	Clinical	-	-	✓	1
Haemophilus influenzae	Fastidious	ATCC49247	-	-	✓	1
Streptococcus pneumoniae	Fastidious	ATCC49619	-	-	✓	1
Candida albicans	Yeast	Clinical	✓	✓	✓	1
Candida glabrata	Yeast	Clinical	✓	✓	✓	1
Cryptococcus neoformans	Yeast	ATCC32045	-	-	✓	1

GN = Gram Negative; GP = Gram Positive.

specimen transport. After incubation, all tubes were briefly vortexed at low speed for three seconds and 10 μL spots were plated in triplicate on either blood agar or chocolate agar for the bacteria, and Sabouraud brain heart infusion agar for the yeasts. After incubation for 24 hours, the resulting cfu/ml were quantified by multiple operators and a total of 9 data points were collected from each set of microorganism vs a specific tube type. The differences between tube-types were determined using pairwise Student's *t* tests. For better comparison, each set of recovered microorganism counts were normalized by the colony count from PNA of that set, so that the recovery from PNA is all adjusted to 1 as baseline.

3. Results

No significant difference in the recovery rate was found in the yeasts and several clinically relevant bacteria between PNA, PCA and GCA (Fig. 1). However, using CSF as dilution matrix, recovery of *H. influenzae* was significantly ($P < 0.001$) reduced in both PCA and GCA compared with the PNA (Fig. 1C), indicating a negative impact of the clot activator. This adverse effect was verified by repeat experiment.

4. Discussion

This study underscores the importance of performing verification studies for off-label uses of specimen collection containers, like CAVs that were not validated for microbial recovery. In this case, although the recovery of most bacteria and yeasts tested in this study did not appear to be affected by the clot activator, the recovery of *H. influenzae* from CSF was found to be significantly reduced by tubes containing clot activator, regardless of glass or plastic material. *H. influenzae* is an important pathogen that can cause serious diseases like meningitis. Therefore, CSF should not be collected in a clot activator-containing tube. One of the limitations of this study is that we only evaluated a limited number of common pathogens that are clinically relevant to the three body fluids most commonly collected in the Vacutainers® in our hospital system, and we did not evaluate the impact of the clot activator on the anaerobic bacteria recovery. Clinical microbiology laboratories should remain vigilant and exercise caution when accepting specimens collected in atypical transport containers for culture, even when reported with a disclaimer.

List of Abbreviations

CAV	Clot activator-containing Vacutainer®
SOP	Standard operating procedure
CLSI	Clinical Laboratory Standards Institute
PCA	plastic clot activator-containing Vacutainer
GCA	glass clot-activator containing Vacutainer
PNA	plastic no additive Vacutainer

Competing interests

The authors declare that they have no competing interests.

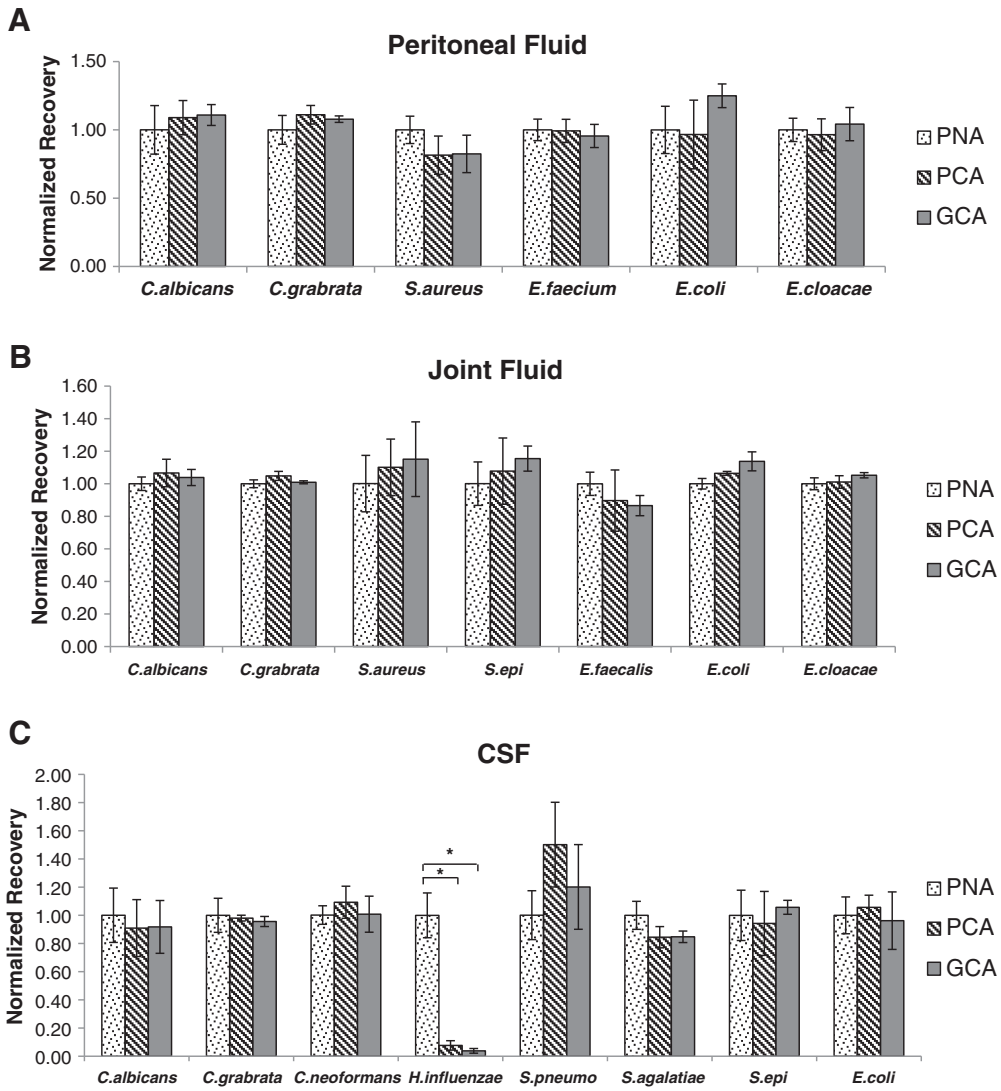

Fig. 1. Quantitative recovery of organisms incubated in each tube type. Bars represent the normalized average recovered cfu/ml from three replicates of each microorganism seeded in

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