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*Contact:
scampeau@axdx.com

Pathogen recovery from bronchoalveolar lavage specimens using an enhanced culture method as compared to standard of care testing

Shelley Campeau[1]*, Blake Buchan[2], Karissa Culbreath[3], Bryan Schmitt[4], David Lyttle[1], Niels Oppermann[1], Kyle Spafford[1], Dayanara Rojas[1], Brooke LeGassick[1], Nico Magnano[1], Romney Humphries[1]

[1]Accelerate Diagnostics, Inc. [2]Medical College of Wisconsin [3]Tricore Reference Laboratories [4]Indiana University School of Medicine

INTRODUCTION

Pneumonia is one of the 10 leading causes of death in the US.[1] Common causative agents of lower respiratory infections caused by bacteria vary slightly depending on whether or not it is community-acquired or nosocomial (either hospital-acquired or ventilator-associated pneumonia, HAP or VAP, respectively) in origin. However, laboratory practices for culturing bronchoalveolar lavage (BAL) specimens, a sample often collected for pneumonia, vary across institutions and often times, the microbiology lab in unaware of the clinical diagnosis/suspicion at the time a specimen is submitted for culture. In addition, methods used to culture BALs can also vary; for instance, some sites perform full quantitation, semi-quantitation, or both; differing test media may be used for routine cultures; and different approaches for selective pathogen reporting may be applied. This study evaluated an enhanced culture method performed at a reference laboratory (RL) as compared to standard of care (SOC) testing at three academic medical center microbiology laboratories for identification of potential pathogens (PP). Additionally, this study looked at the reportability of a subset of PP based on each site's standard operating procedure (SOP) to determine differential reporting practices, if any, among the three sites.

[1]Heron M. Deaths: leading causes for 2016. *Natl Vital Stat Rep* 2018;67 (6).

METHODS

- 162 BAL specimens were cultured at 3 clinical laboratories by their respective standard of care (SOC) and sent, refrigerated, to Accelerate Diagnostics, Inc. reference lab (RL) where they were plated upon receipt and read at 24 and 72 hours (h):
  - Site 1 performed quantitative culture (1 µl and 10 µl loops) only on BAP, CHOC, MAC, CNA, HIA*
  - Site 2 performed quantitative (1 µl and 10 µl loops) and/or semi-quantitative cultures on BAP, CHOC, MAC, CNA, depending on order
  - Site 3 performed quantitative (1 µl and 10 µl loops are split on extra BAP and MAC plates) and semi-quantitative cultures on BAP, CHOC, MAC, CNA
  - RL performed semi-quantitative culture on BAP, CHOC, MAC, HIA, SSA, CET, and CHROM using 1 µl loop and an extra BAP using a 10 µl loop
- Herein, "routine" media refers to BAP, CHOC, MAC, and CNA and "supplemental" media refers to HIA, SSA, CET, and CHROM.
- Identification at each laboratory was performed according to their respective SOC method and isolates considered to be clinically significant were recorded in an electronic database capture system, using the below categories. Clinical significance was defined by whether or not a site performed and reported a complete identification and/or susceptibility testing.
  - 1 = 1+ (1-10 colonies; $<10^3$)
  - 2 = 2+ (11-99 colonies; 10^3-10^4)
  - 3 = 3+ (100-1000 colonies; 10^4-10^5)
  - 4 = 4+ (confluent growth; $>10^5$)
- At the RL, all organism morphologies were semi-quantitated and identified using MALDI-TOF MS.
- The potential pathogen (PP) reported by SOC and RL were compared.
- PP discrepancies between SOC and/or RL, sites were asked to provide details for isolates that might have grown on SOC culture but not reported because they were deemed insignificant, as defined by their SOPs.

*BAP = Tryptic soy agar with 5% sheep's blood; CHOC = Chocolate agar; MAC = MacConkey; CNA = Columbia colistin nalidixic acid; HIA = Haemophilus isolation agar; SSA = Streptococcus selective agar; CET = cetrimide; CHROM = CHROMagar S. aureus

RESULTS

RESULTS – PP RECOVERY

46 out of 162 (28.4%) specimens had at least 1 PP identified by SOC and/or the RL:

- *Acinetobacter baumannii* (1); *Citrobacter,* CIT (3); *Enterobacter* (5); *E. coli,* ECO (3); *Haemophilus influenzae* (2); *Klebsiella* (6); *Proteus,* PRO (4); *Pseudomonas aeruginosa,* PAE (14); *Serratia marcescens,* SMA (4); *Staphylococcus aureus,* SAU (13); *Streptococcus pneumoniae,* SPN (5); *Stenotrophomonas maltophilia,* STM (7).

Among the 46 specimens, 67 PP were identified by both SOC and RL, SOC only, or RL only (**Figure panel A**).

Of the 67 PP identified, a breakdown of organisms is detailed in **Figure panel B** and the **Table**:

- 42 (62.7%) were reported by both SOC and RL
- 9 (13.4%) by SOC and not RL
  - All PP missed by the RL were present at ≤ 2+, except for 1 (3+) in a specimen with 7 PP
- 16 (23.9%) by the RL and not SOC
  - PP missed by SOC were present at 1+ to 4+ quantity
  - One specimen detected by RL only was determined to likely be a transcriptional error but could not be resolved. It is not included in the table
- No trend in pathogen recovery was identified across the different SOC laboratories.

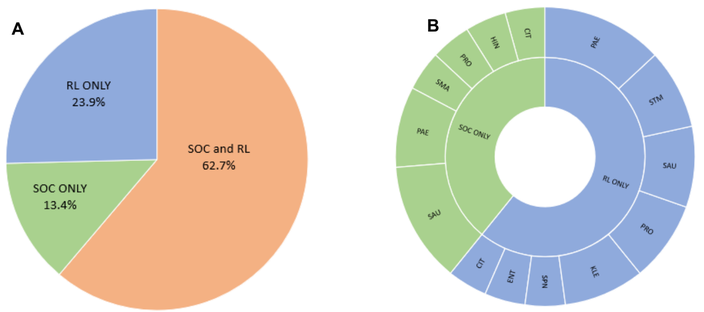

Figure. PP recovery by SOC and RL, SOC only, and RL only **(A)**. Breakdown of PP from SOC only and RL only **(B)**. HIN, *Haemophilus influenzae.*

RESULTS – DIFFERENTIAL REPORTING

For the 24 discrepant PP recovered by only by SOC or RL (excluding the unresolved specimen), the predicted reporting of each PP based on each site's standard operating procedures for lower respiratory tract cultures is shown in the **Table** (shaded boxes indicate the enrolling site for the specimen with the PP).

- 16 (66.7%) of PP would have been reported differentially across the 3 sites
- 8 (33.3%) of PP would have been reported the same across all 3 sites

Table. Summary of PP recovered by only SOC or RL.

Site sample #	PP detected	PP amount	NOF amount	Did supplemental media improve recovery or ID? (media)	Would be identified by Site's SOP: Site 1	Site 2	Site 3	Additional organisms recovered at enrolling site
PP Recovered by SOC Only								
01-0017	*H. influenzae*	2	2	YES (HIA)	NO *exception	YES	NO	1 PP 4+ *Reported by site
01-0022	*P. mirabilis*	3	None	YES (HIA)	YES	NO	YES	6 PP ranging 2+ to 3+
	S. marcescens	3		NO	YES	NO	YES	
01-0030	*C. freundii*	1	1	NO	YES	NO	NO	1 PP (KLE) 2+ 1 non-PP 3+
03-0005	*P. aeruginosa*	2	1	N/A	YES	YES	YES	
03-0020	*P. aeruginosa*	2	1	N/A	YES	YES	YES	
03-0056	*S. aureus*	1	1	N/A	YES	YES	NO *exception	1 PP 4+ *Reported by site
03-0059	*S. aureus*	1	None	N/A	NO	NO	YES	1 PP 3+
03-0067	*S. aureus*	1	1	N/A	NO	YES	YES	
PP Recovered by RL Only								
01-0003	*P. mirabilis*	2	1	YES (HIA, SSA)	NO	YES	YES	1 PP 4+
01-0006	*P. aeruginosa*	1	None	NO	NO	NO	NO	
01-0008	*K. oxytoca*	1	None	YES (HIA)	NO	NO	YES	No growth reported
01-0015	*C. koseri*	4	4	NO	NO	YES	YES	NOF 4+
01-0020	*P. aeruginosa*	1	2	YES (CET)	NO	NO	NO	2 PP ranging from 2+ to 3+
01-0025	*E. cloacae*	1	3	NO	NO	YES	YES	1 non-PP 3+
02-0007	*S. maltophilia*	1	2	YES (SSA)	NO	NO	NO	
02-0008	*S. pneumoniae*	1	1	YES (SSA)	NO	NO	NO	
02-0029	*S. aureus*	2	2	YES (SSA)	NO	YES* exception	NO	*Unable to resolve discrepancy with site
02-0037	*S. aureus*	4	4	YES (SSA)	NO	YES* exception	NO	*Unable to resolve discrepancy site
03-0011	*S. maltophilia*	2	3	YES (SSA)	NO	NO	NO	4 PP ranging from 2+ to 3+ 1 non-PP 3+ NOF 3+
03-0045	*S. maltophilia*	1	None	NO	NO	NO	NO	No growth
03-0054	*K. oxytoca*	1	4	YES (HIA)	NO	YES	NO	
	P. aeruginosa			YES (CET)	NO	NO	NO	
03-0066	*P. mirabilis*	1	3	YES (SSA)	NO	YES	NO	1 PP 2+

CONCLUSIONS

- The majority of PP identified by SOC were identified by the RL, despite 48 h refrigerated transport.
- Supplemental media improved detection/recovery for 13 (54%) of discrepant PP recovered from SOC or RL.
- Differential reporting across sites may also lead to inconsistencies of PP reporting.