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Automated Removal of Inhibitory Substances from Bronchoalveolar Lavage Specimens

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INTRODUCTION

Bronchoalveolar lavage (BAL) and mini-BAL specimens are used in the diagnosis of lower respiratory tract infections. However, the presence of inhibitory substances in these specimens, such as antibiotics, inhaled steroids, hemolyzed blood, mucin, or lidocaine, can hinder detection of pathogens present in low concentrations.

The Accelerate PhenoPrep™ module (AXDX) performs automated specimen cleanup prior to loading on the Accelerate Pheno™ system, which then provides identification (ID) and MIC-based phenotypic antimicrobial susceptibility testing (AST) results direct from the specimen. This study measured the ability of AXDX to remove inhibitory substances from respiratory BAL and mini-BAL specimens.

METHODS

Fresh BAL and Mini-BAL Specimens

80 fresh positive BAL or mini-BAL specimens from 6 clinical sites were tested. A diffusion screening method was developed to detect the presence of inhibitory substances, which was performed before and after specimen processing using AXDX. Briefly, 100 µL of BAL specimen was pipetted directly onto the center of 4 Mueller-Hinton agar plates and allowed to dry for 4 hours. These plates were then inoculated with *S. aureus* ATCC® 29213 (SAUR), *E. coli* ATCC® 25922 (ECOL), *P. mirabilis* IHMA 827374 (PRMI), and *P. vulgaris* ATCC® 6380 (PROV). Plates were incubated 18-24 hours at 35ºC, and both the pre- and post-processed specimen zones of inhibition were recorded in millimeters (**Figure 1**). Pre- and post-prep aliquots of samples with inhibition were then combined 1:1 with Mueller Hinton broth and left to grow for 4 hours to determine whether more bacteria would grow from samples after inhibitory substances were removed.

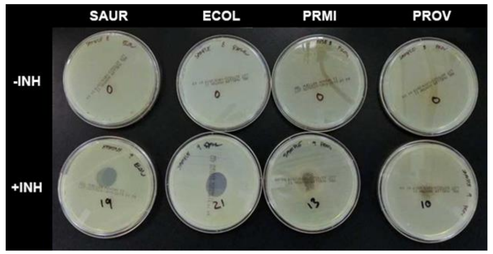

Figure 1. Examples of BAL specimens with (+INH) and without (-INH) inhibition using the diffusion screening method.

Contrived Specimens with Known Inhibitory Substances

In addition, known concentrations of various inhibitory substances present at relevant levels in respiratory BAL fluid were contrived into artificial respiratory matrix designed to mimic BAL specimens and processed with AXDX. The diffusion screening method was used to measure inhibition prior to and after AXDX processing using SAUR and ECOL as described above as well as additional organisms: *A. baumannii*® ATCC 19606 (ABAU), *H. influenzae* ATCC® 43334 (HINF), *P. aeruginosa* ATCC® 27853 (PSAR), *S. maltophilia* ATCC® 49130 (STMA) and *S. pneumoniae* ATCC® 49619 (STPN).

RESULTS – Fresh Specimens

Table 1. All BAL and mini-BAL specimens exhibiting inhibition prior to sample processing had inhibition removed post-processing.

Site	Specimen Number	Pre-Processing Zone of Inhibition (mm)				Post-Processing Zone of Inhibition (mm)			
		SAUR	ECOL	PRMI	PROV	SAUR	ECOL	PRMI	PROV
1	3437	0	0	12	0	0	0	0	0
1	3568	0	7	0	15	0	0	0	0
1	3569	0	0	13	17	0	0	0	0
1	3618	22	0	0	0	0	0	0	0
1	3662	28	0	36	36	0	0	0	0
1	3887	0	12	17	17	0	0	0	0
1	3937	0	20	20	15	0	0	0	0
1	3941	4	28	34	22	0	0	0	0
1	3966	0	0	0	5	0	0	0	0
2	619	0	0	24	27	0	0	0	0
2	620	6	13	0	0	0	0	0	0
2	628	8	0	12	15	0	0	0	0
2	644	14	25	32	32	0	0	0	0
2	656	0	12	0	0	0	0	0	0
2	658	0	14	12	7	0	0	0	0
3	1110	19	21	13	10	0	0	0	0
3	1244	15	0	17	0	0	0	0	0
3	1714	0	25	44	37	0	0	0	0
3	1720	0	0	29	22	0	0	0	0
3	1748	0	13	37	20	0	0	0	0
4	105	0	18	35	31	0	0	0	0

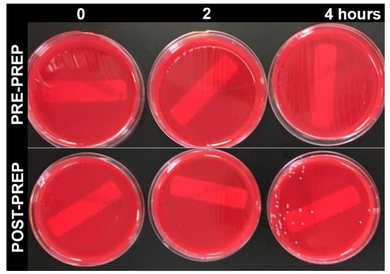

Figure 2. Example of a specimen from site 1 (3927) exhibiting inhibition before (pre-prep) and growth after (post-prep) specimen processing using AXDX.

Table 1 shows that out of 80 fresh positive specimens, 21 (26%) exhibited inhibition prior to AXDX processing, with zones of inhibition ranging from 4 to 44 mm across the different plated organisms. After processing, all zones of inhibition were 0 mm, indicating the inhibitory substance(s) were removed. Additionally, **Figure 2** shows a representative sample exhibiting no growth before, but growth following processing with AXDX. This indicates that inhibitory substances present in the sample were hindering growth.

RESULTS – Contrived Specimens

Table 2. Inhibition in contrived samples prior to sample processing.

Interferent	Concentration Tested	Pre-Processing Zone of Inhibition (mm)						
		ABAU	ECOL	HINF	PSAR	SAUR	STMA	STPN
Aztreonam	18.7 µg/mL	0	0	0	0	0	0	0
Blood	10% v/v	0	0	0	0	0	0	0
Ceftriaxone	1.14 µg/mL	0	0	0	0	0	0	0
Ertapenem	10.8 µg/mL	0	28	32	0	23	0	26
Ephedrine	0.012 µg/mL	0	0	0	0	0	0	0
Gentamicin	4.66 µg/mL	0	0	19	0	13	0	0
Guaifenesin	2.9 µg/mL	0	0	0	0	0	0	0
Levofloxacin	51.6 µg/mL	0	0	35	39	0	33	29
Lidocaine HCl	30% v/v	0	0	0	0	0	0	0
Linezolid	97.4 µg/mL	0	0	34	0	38	0	35
Mucin	20,000 µg/mL	0	0	0	0	0	0	0
Nebulizing NaCl solution	5% v/v from a 3% NaCl solution	0	0	0	0	0	0	0
Pentamidine	0.2055 µg/mL	0	0	0	0	0	0	0
Trimethoprim/ Sulfamethoxazole (Co-trimoxazole)	13.6 µg/mL/ 372 µg/mL	19	0	28	0	45	45	19
Vancomycin	6.8 µg/mL	0	0	0	0	11	0	19

Table 2 shows that out of the 15 interferents tested at relevant concentrations which may be present in the lung, 6 (40%) exhibited inhibition prior to AXDX processing, with zones of inhibition ranging from 11 to 45 mm. Of the known interferents tested, only some of the antibiotics resulted in inhibition prior to sample processing. After processing, all zones of inhibition were 0 mm, indicating the inhibitory substance was removed.

CONCLUSIONS

These results indicate that the Accelerate PhenoPrep™ module successfully removes inhibitory substances from BAL specimens, which can aid in the detection and AST of low concentration organisms when used in conjunction with the Accelerate Pheno™ system.