Direct Identification of Industry Relevant Bacteria and Yeast from Positive BacT/ALERT® Media Using the VITEK® MS

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INTRODUCTION

Combining automated methods for microorganism detection and identification is ideal for industry customers looking to perform the least number of processing steps required to classify a contamination event with rapid turnaround and high confidence in results. The BacT/ALERT® 3D Systems (BTA) and VITEK® MS (VMS) instrument provide an alternative to more labor intensive compendial detection and identification methods, respectively. A study was performed to assess the ability of the VMS to correctly identify microbial isolates recovered in BTA media.

MATERIALS AND METHODS

Fifteen isolates (BioBall™ or culture) representing USP/EP/JP Sterility Test chapter and environmental microorganisms were examined in this study. See Table 1.

BacT/ALERT® Sample Preparation:
BioBall MultiShot-550 strains were prepared per manufacturer's instructions in Rehydration Fluid and diluted to ≤ 100 cfu/mL in phosphate buffered saline (PBS). Cultured microorganisms were prepared to a 1.0 McFarland turbidity and diluted 10^{-7} in PBS to an estimated ≤ 100 cfu/mL. Microorganisms were inoculated in 0.5 mL quantities (approximately 50 cfu) into each of five replicate BTA bottles and were incubated at 32.5˚C until positive. Positive bottles were removed from the instrument in log or early stationary phase and allowed to come to room temperature. The bottle septum was swabbed with a 70% alcohol pad and the bottle vented with a subculture unit (BMX P/N 233766) to relieve pressure.

VITEK® MS Sample Preparation:
Samples of 1.5 mL were drawn from each bottle using a 18-G needle attached to a syringe, transferred to an Eppendorf tube and centrifuged for 1 min at 10,000 x g. The supernatant was discarded, the pellet washed with 1 mL sterile water and vortexed to resuspend. The samples were centrifuged again for 1 min at 10,000 x g. The supernatant was discarded and 100 µL 70% ethanol was added to resuspend the pellet.

Depending on turbidity of suspension, 0.5 - 1.0 µL was spotted in quadruplicate on the target slide for each of the five replicate BTA bottles. Prior to the sample drying on the slide, 1 µL of CHCA for bacteria or 0.5 µL FA Reagent for yeast was added to the spot. Bacterial samples were allowed to dry prior to processing. For yeast, the FA Reagent was allowed to dry prior to adding 1 µL CHCA and then left to dry. Via the Prep Station, the correct algorithm (Bacterial or Fungal) was assigned to the sample. The slides were loaded into the VMS and allowed to process.

RESULTS AND DISCUSSION

All microorganism / BTA bottle combination replicates were positive as determined by the BTA bottle algorithm. Positive bottles were removed from the incubator between log and early stationary growth phase ensuring that the biomass of the sample was sufficient.

Each bottle was prepared and spotted onto the VMS slide as stated in the Materials and Methods section. Replicate testing of the ethanol sample preparation was conducted to account for variability in sampling or settling of the biomass in ethanol. A total of 20 spots for each microorganism (5 BTA replicates x 4 spots each) were prepared. Instances of less than 20 total spots indicate that contamination determined by subculture (data not shown) was observed. See Table 1. The VMS replicate performance column lists the proportion of correct identification. Instances of less than 100% indicates results of 'No Identification' or 'Bad Spectrum During Acquisition' when the analysis is compared to the database. This may be due to sampling and repeat testing on the same sample was not conducted. It is recommended that preparation of multiple spots be conducted to ensure appropriate sampling.

For *C. albicans* NCPF 3179, the biomass was present in small tufts and was unable to be drawn from the in iFA Plus medium. It was therefore not tested on the VMS.

Table 1. VMS Identification Results of Industry Relevant Microorganisms Sampled from Positive BTA Media

Microorganism	BTA Bottle Type	Mean TTD (Days)	VITEK MS result (Version IVD 2.0)	Replicate Performance	Highest Confidence Achieved
Aerobic Microorganisms					
Bacillus subtilis NCTC 10400	iAST	0.74	*Bacillus subtilis / Bacillus amyloliquefaciens*	18 / 20	50.0% / 50.0%
	iFA Plus	0.75	*Bacillus subtilis / Bacillus amyloliquefaciens*	16 / 20	50.0% / 50.0%
Candida albicans ATCC 10231™	iAST	1.09	*Candida albicans*	15 / 16	99.9%
	iFA Plus	1.23	*Candida albicans*	15 / 16	99.9%
Candida albicans NCPF 3179	iAST	1.23	*Candida albicans*	20 / 20	99.9%
Escherichia coli ATCC 8739™	iAST	0.64	*Escherichia coli*	20 / 20	99.9%
	iFA Plus	0.64	*Escherichia coli*	20 / 20	99.9%
Micrococcus luteus BMX 17909	iAST	2.10	*Micrococcus luteus / lylae*	19 / 20	99.9%
	iFA Plus	4.40	*Micrococcus luteus / lylae*	11 / 20	99.9%
Micrococcus luteus BMX 17910	iAST	2.70	*Micrococcus luteus / lylae*	12 / 12	99.9%
	iFA Plus	2.50	*Micrococcus luteus / lylae*	3 / 8	99.9%
Pseudomonas aeruginosa NCTC 12924	iAST	0.91	*Pseudomonas aeruginosa*	19 / 20	99.9%
	iFA Plus	0.93	*Pseudomonas aeruginosa*	19 / 20	99.9%
Staphylococcus aureus NCTC 10788	iAST	1.10	*Staphylococcus aureus*	20 / 20	99.9%
	iFA Plus	0.85	*Staphylococcus aureus*	10 / 10	99.9%
Yersinia enterocolitica ATCC 9610™	iAST	0.98	*Yersinia enterocolitica*	20 / 20	99.9%
	iFA Plus	1.03	*Yersinia enterocolitica*	20 / 20	99.9%
Anaerobic and Facultative Microorganisms					
Bacteroides fragilis ATCC 25285™	iNST	1.54	*Bacteroides fragilis*	20 / 20	99.9%
	iFN Plus	2.08	*Bacteroides fragilis*	20 / 20	99.9%
Bacteroides vulgatus ATCC 8482™	iNST	2.60	*Bacteroides vulgatus*	18 / 20	99.9%
Clostridium sporogenes ATCC 11437™	iNST	1.10	*Clostridium sporogenes*	18 / 20	99.9%
	iFN Plus	1.39	*Clostridium sporogenes*	16 / 20	99.9%
Clostridium sporogenes NCTC 12935	iNST	0.92	*Clostridium sporogenes*	20 / 20	99.9%
	iFN Plus	1.05	*Clostridium sporogenes*	11 / 16	99.9%
Escherichia coli ATCC 8739™	iNST	0.56	*Escherichia coli*	20 / 20	99.9%
	iFN Plus	0.61	*Escherichia coli*	20 / 20	99.9%
Propionibacterium acnes ATCC 11827™	iNST	4.90	*Propionibacterium acnes*	20 / 20	99.9%
	iFN Plus	7.22	*Propionibacterium acnes*	8 / 20	99.9%
Propionibacterium acnes DSM 1897	iNST	5.20	*Propionibacterium acnes*	20 / 20	99.9%
	iFN Plus	9.76	*Propionibacterium acnes*	20 /20	99.9%
Staphylococcus aureus NCTC 10788	iNST	0.84	*Staphylococcus aureus*	20 / 20	99.9%
	iFN Plus	1.13	*Staphylococcus aureus*	20 / 20	99.9%
Yersinia enterocolitica ATCC 9610™	iNST	0.99	*Yersinia enterocolitica*	20 / 20	99.9%
	iFN Plus	1.05	*Yersinia enterocolitica*	20 / 20	99.9%

Table 2. Estimated Sample Processing Turn Around Time

Time Savings from positive BTA result (1 positive result) to Identification:			
Sample processing work flow (this method)		**Sample processing work flow (standard method)**	
Bottle prep through ethanol suspension	5 min	Bottle prep / subculture	< 5 min
Prep VMS slide (4 replicates), Prep Station entry	5 min	Incubation for initial growth	18 - 24 h
Load and run VMS	5 - 7 min	Identification Method	Varies
Total time to result	**< 20 min**	**Total time to result**	**> 1 d**

For *S. aureus* in iFA Plus medium, repeat testing to assess a 0.5 µL spot for the VMS was conducted and compared to a 1.0 µL spot. A higher confidence and a greater propensity for correct replicate identification was observed when less biomass (e.g., 0.5 µL) was spotted. In subsequent testing, either 0.5 µL or 1.0 µL sample spots were examined. The volume chosen was based on observation of the ethanol suspension turbidity. A milky sample was tested using the smaller volume.

Further method development may be assessed by examining the biomass directly from the positive BTA bottle. Preliminary studies indicated that diluting the BTA culture to an approximate 4.0 McFarland and testing 1.0 µL per spot on the VMS provided correct identification with a high level of confidence.

For *P. acnes* ATCC 11827™, the biomass was insufficient despite a positive BTA result. Although the VMS was able to identify this species, only 8 of 20 replicates provided acceptable results. No repeat testing was conducted.

From Table 2, combination of BTA and VMS for microbial detection and identification results in a faster turnaround time compared to the standard subculture method. This allows the industrial facility to perform OOS investigations and more rapidly assess the quality of their product.

The ability of the BacT/ALERT culture media to detect a wide variety of microorganisms coupled with the speed of identification provided by the VITEK MS create a reliable and rapid method of identifying contamination.

CONCLUSIONS

- **The proposed method used for microbial identification from positive BacT/ALERT® media without subculture using the VITEK® MS provides a rapid and reliable result with a high degree of confidence.**
- **Coupling the BacT/ALERT® and VITEK® MS provides a rapid, time-saving method for identification of contamination.**