Paper # **C-5**

Preliminary Development and Verification of a New Agitation Method for the ESP Culture Technology

N. M. SULLIVAN and D. FETT

TREK Diagnostic Systems, Inc., Westlake, OH

25760 First Street
Westlake, Ohio 44145
800.871.8909 • www.trekds.com

ABSTRACT

The next generation of ESP Culture System (VersaTREK™) by TREK Diagnostic Systems, Westlake, OH has been developed with a new method of agitation (Vortrexing™) for aerobic cultures. Because the efficiency of agitation is critical to the growth and signal of aerobic organisms, the new method was evaluated to determine its compatibility with the ESP technology and its performance compared to current agitation method. Both signal quality and time to detection were evaluated as performance parameters. After investigating several methods of agitation, a stir bar method was further developed for use within the VersaTREK instrument. The first experiments included determination of the optimal revolutions per minute (rpm) of the stir bar using four strict aerobic microorganisms. The organisms were *Stenotrophomonas maltophilia, Acinetobacter haemolyticus, Neisseria meningitidis*, and *Haemophilus influenzae*. After the optimum rpm range was determined, further studies were done with both 80 and 40-ml bottles with 25 clinical and stock microbial strains, which included yeast, facultative anaerobes, and strict aerobic bacteria. Many of the organisms in the study were known to require oxygen for growth or signal generation, thus were considered "worst-case". Results from these performance tests showed that the new stir bar agitation was better than or equal to the rotational method in time to detection and, the quality of the oxygen consumption curve was better with the new method. The conclusion from these preliminary studies is that the stir bar method is compatible with the ESP technology generating the same or better performance compared to the current ESP agitation method. A major advantage of the stir bar method over the current ESP system is that agitation can be activated at any location and does not require placement of aerobic and anaerobic bottle sets into separate physical sections of the instrument. Clinical trials of the new instrument with this new agitation method will be done as a final step in validation of performance.

INTRODUCTION

The ESP Culture System II was first commercialized in October of 1992(1). The detection technology still remains unique to this day. Instead of a restricted CO2-based detection technology, ESP utilizes a transducer that detects head space pressure changes that is a result of microbial growth (1). For the past 10 years, several system improvements have been done for reliability and better performance, but nothing was done to address some of the workflow and ergonomic improvements people have suggested throughout the years.

TREK set out this past year to design a new instrument, VersaTREK (Figure 1), that improved the areas of ergonomics, reliability, and workflow. The first step in the development of the new instrumentation was to evaluate an new method of agitation for aerobic cultures. The goal was to give the instrument more versatility than the current ESP has in assignment and use of test locations. The ability to place any bottle anywhere was the desired goal, instead of the current ESP instrument that physically separates the aerobic and anaerobic bottles in the unit. The other areas of design improvements include bottle management, entry of test bottles, easy one-step access to bottle information, and remote diagnostics.

The first studies included evaluations of several agitation methods, such as ultrasound and tapping using strict aerobic organisms. After evaluations of these agitation methods, the stir bar method (Vortrexing, Figure 2) was selected for further development. This poster presents the data from the initial prototype instrument using stir bar agitation and compares the results to the ESP Culture System II.

MATERIALS & METHODS

Microorganisms:

Table 1.The Microorganisms used in the studies were ATCC® stock strains and clinical strains.

Strain	Strain Number	Strain	Strain Number
Acinetobacter baumannii	ATCC 19606	H. parainfluenzae	ATCC 7901
A. haemolyticus	ATCC 19002	Kingella kingae	ATCC 23330
Bordetella pertussis	ATCC 8467	Klebsiella pneumoniae	ATCC 23357
Candida albicans	ATCC 18804	Neisseria gonorrhoeae	SJH 9070
C. glabrata(Torulopsis)	Dif 6822	N. meningitidis	ATCC 13090
C. krusei	ATCC 6258	Pseudomonas aeruginosa	ATCC 27853
C. parapsilosis	ATCC 34136	Staphylococcus aureus	ATCC 25923
Cardiobacterium hominis	Stanford 253	S. capitis	ATCC 7815
Corynebacterium jeikeium	ATCC 43217	S. epidermidis	BC 2864
Enterococcus faecalis	ATCC 19433	S. epidermidis	ATCC 14990
Escherichia coli	ATCC 25922	Streptococcus pneumoniae	ATCC 6303
Haemophilus actinomycetemcomitans(Actinobacillus)	ATCC 29523	S. pneumoniae	ATCC 6305
H. influenzae	ATCC 19418	Stenotrophomonas maltophilia	ATCC 17672

Inoculation of test bottles:

1. Organisms were grown from frozen stock and streaked onto blood or chocolate agar for 24-72 hours.
2. The organisms were diluted to 10-100 organisms in 0.5-1.0 ml in sterile saline
3. The bottles were inoculated with the organisms and blood where necessary. 0.5 to 2.5 ml of blood was added to test bottles containing *Haemophilus* sp., *Bordetella*, and *Neisseria gonorrhoeae*. All other strains did not require supplementation for growth and signal.
4. The test bottles were placed on the respective test instruments: ESP culture system II agitated and non-agitated, VersaTREK prototype.
5. The bottles were left on the instrument for the entire curve generation in order to determine curve quality.

Reproducibility experiments:

To show reproducibility of the agitation method, *Neisseria meningitidis* was inoculated to multiple bottles tested on three separate days and placed onto the VersaTREK prototype. Time to detection was recorded.

Figure 1. New instrument design- VersaTREK

Figure 2. Stir bar agitation in the new instrument – Vortrexing

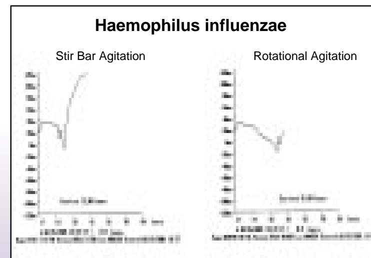

Figure 3. Resulting graphs from the Stir bar agitation and the current ESP II agitation for Haemophilus influenzae.

RESULTS

Table 2. Reproducibility of the Vortrexing agitation method in the VersaTREK prototype with N. meningitidis. Experiments performed on three different days are shown.

Time to detection 80 ml		Time to detection 40 ml	
18.2	16.8	16.8	16.6
17.8	17.0	17.4	17.2
17.0	NP[a]	17.0	17.0
17.4	NP[a]	17.4	17.4
17.2	17.0	17.2	16.6
17.2	17.2	17.2	16.8
17.4	16.8	17.2	16.0
17.4	16.2	17.2	16.8

a - NP = No pair bottle

Table 3. Results from 25 test strains comparing ESP II (current) and VersaTREK (stir bar) agitation in 80 ml bottles[a].

Test strain	Time to detection		
	VersaTREK	ESP II agitated	ESP II non agitated
Acinetobacter baumannii	13.8	14.6	25.4
Acinetobacter haemolyticus	11.6	11.8	24.4
Bordetella pertussis	69.4	79.2	NS[b]
Candida albicans	21.4	22.0	24.6
Candida glabrata	19.2	18.6	21.6
Candida krusei	24.8	26.4	46.4
Candida parapsilosis	25.0	23.6	NS
Cardiobacterium hominis	35.6	38.4	NS
Corynebacterium jeikeium	27.6	30.2	68.6
Enterococcus faecalis	11.0	12.8	13.4
Escherichia coli	6.8	8.8	11.6
Haemophilus actinomycetemcomitans	21.2	26.4	27.6
Haemophilus influenzae	17.0	35.8	NS
Haemophilus parainfluenzae	16.7	17.0	NS
Kingella kingae	16.2	16.4	NS
Klebsiella pneumoniae	11.8	12.0	13.8
Neiserria gonorrhoeae	33.6	32.0	NS
Neiserria meningitidis	17.0	18.2	NS
Pseudomonas aeruginosa	15.8	17.2	22.2
Staphylococcus aureus	10.8	11.8	15.2
Staphylococcus capitis	25.0	31.0	37.4
Staphylococcus epidermidis BC 2864	15.8	16.2	22.2
Staphylococcus epidermidis 14990	15.6	16.2	21.8
Stenotrophomonas. maltophilia	19.6	18.6	25.2
Streptococcus pneumoniae 6303	15.8	18.2	17.8
Streptococcus pneumoniae 6305	16.4	15.2	23.0

a- The stir bar tests with times to detection that differ greater than 2 hours are in red.
b- NS – No Signal

RESULTS con't

Table 4. Results from 25 test strains comparing ESP II (current) and VersaTREK (stir bar) agitation in 40 ml bottles[a].

Test strain	Time to detection		
	VersaTREK	ESP II agitated	ESP II non agitated
Acinetobacter baumannii	14.4	17.0	22.6
Acinetobacter haemolyticus	11.6	11.8	14.8
Bordetella pertussis	70.3	79.4	NS[b]
Candida albicans	21.0	22.0	22.6
Candida glabrata	16.4	18.6	NT[c]
Candida krusei	24.4	26.2	32.8
Candida parapsilosis	23.4	40.6	NS
Cardiobacterium hominis	24.4	26.2	32.8
Corynebacterium jeikeium	26.0	30.2	NS
Enterococcus faecalis	11.8	12.4	12.6
Escherichia coli	6.8	8.8	11.6
Haemophilus actinomycetemcomitans	22.8	22.0	NT
Haemophilus influenzae	17.6	37.0	NS
Haemophilus parainfluenzae	14.4	16.6	NS
Kingella kingae	16.6	18.4	NS
Klebsiella pneumoniae	11.6	11.4	12.8
Neiserria gonorrhoeae	26.8	28.2	NS
Neiserria meningitidis	17.0	17.2	NS
Pseudomonas aeruginosa	21.0	22.0	22.6
Staphylococcus aureus	13.2	14.0	17.0
Staphylococcus capitis	24.0	29.6	31.4
Staphylococcus epidermidis BC 2864	15.8	20.6	22.2
Staphylococcus epidermidis 14990	13.8	15.6	21.8
Stenotrophomonas. maltophilia	19.0	18.8	23.0
Streptococcus pneumoniae 6303	13.8	18.2	17.8
Streptococcus pneumoniae 6305	12.2	15.2	25.8

a- The tests with times to detection greater than 2 hours faster for the stir bar method are in red.
b- NS – No Signal
c- NS – Not Tested

DISCUSSION AND CONCLUSION

Table 2 shows the reproducibility of the stir bar agitation with *N. meningitidis*. Between and amongst the experiments there was not more than 2 hours difference in time to detection. Within an experiment, there was no more than 1.2 h of a difference in time to detection. The inoculum for these experiments was 10-100 cfu/bottle.

The performance of the stir bar agitation with 25 organisms is shown in tables 3 and 4. The tests with times to detection greater than 2 hours faster for the stir bar are in red. Table 3 shows the results from the 80 ml bottle and Table 4 shows results for the 40 ml bottle. Most responses of the stir bar compared to ESP II were within 2 hours of each other. However, in the 80 ml bottle, eight of 26 organisms were more than 2 hours faster with the stir bar, whereas none were two hours faster with the rotational agitation. In the 40 ml bottle 13 organisms were faster with the stir bar agitation. Of note is the time to detection of *H. influenzae* and *B. pertussis* in both the 80 and 40 ml bottles. The time to detection with the stir bar was 9-18 h faster compared to the ESP II agitation. The main reason for the faster response is the quality of the oxygen consumption curve, allowing the signal to occur faster (Figure 3).

These data show that the stir bar agitation method is a very effective method when used with the ESP technology. The time to detection is the same or better for all isolates tested compared to the current agitation method.

The major advantages of the new stir bar agitation are: 1) the efficient mixing allowing for good oxygenation of the media, which results in better quality of signal, and 2) the ability to place any bottle anywhere in the system. The current ESP System physically separates the aerobic from the anaerobic broths. The aerobic section agitates in the bottom part of the instrument, whereas the anaerobic section is stationary and is in the top part of the instrument.

REFERENCES

1. Sullivan, N.M.., L.K. Tuck, G.S. Welty, and R. Firstenberg-Eden. 1992 Evaluation of a new automated blood culture system. Presented at the Annual meeting of the American Society for Microbiology, New Orleans.