ECCMID 2017
April 22-25, 2017
Vienna, Austria
EV0159

An Automated Sample Preparation Instrument to Accelerate Positive Blood Cultures Microbial Identification by MALDI-Tof mass spectrometry (Vitek®MS)

P. Broyer[1], N. Perrot[1], H. Rostaing[1], J. Blaze[1], F. Pinston[1], M.H Charles[1], G. Gervasi[1], O. Dauwalder[2], F. Vandenesch[2], H. Meugnier[2]
[1]bioMerieux, Innovation Biology & Technology Units, France. [2]Hospices Civils de Lyon, CPBE Laboratory, Lyon, France

Research Study *– Method, Consumables & Instrument not commercially available*

Introduction

Direct microorganism ID from positive blood cultures (BC) by MALDI-TOF Mass spectrometry was a significant progress. However, all the published direct protocols are still manual and time consuming, they require dedicated technician availability, specific management organization and are generally performed by batches. We present a small bench-top instrument for automated preparation of Vitek®MS slide, within 25min, directly from positive BC, based on an "all-in-one" extraction strip, covering the missing links between BacT/ALERT® and Vitek®MS systems.

Materials and Methods

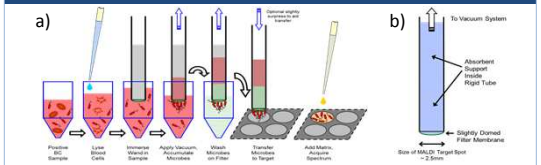

Figure 1. (a) "Filter Wand" Extraction and Spotting principle (*patent W02013/016211. Walsh et al*). After selective RBC lysis, the microorganisms are concentrated and washed at the end of the Filter Wand (FW) disposable. The FW is then used to transfer the microbial mass onto the MALDI spots. (b) Filter Wand schematic description.

Well#1: Positive Blood Culture (1mL)
Well#2: Lysis buffer (Brij97, CAPS, pH 11.7)
Well#3 : Wash buffer 1 (Brij97, NaCl, pH7.2)
Well#4 : Wash buffer 1
Well#5 : Wash buffer 2 (Deionized water)

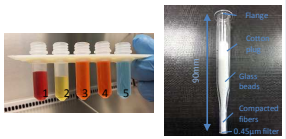

Figure 2. Disposables. Left, the extraction disposable strip : Pre-filled tubes snap into a 3D printed support. Right : Injection molded PS Crystal Filter Wand assembled with Pall 0.45µm SuporR membrane and filled with fibers and glass beads (300µm)

Seeded Blood Cultures : BacT/Alert SA, SN, FA, FN bottles inoculated with 10mL human blood and seeded with ±400 CFU of microorganims. A total of 132 inoculated bottles were loaded into BacT/alert 3D and Virtuo™ blood culture systems .

Clinical Blood Cultures : 92 clinical BC were processed using the BC prep station prototype during the field testing in Lyon HCL Hospital (one month) and were compared with the reference procedure (sub-culture + ID on Vitek®MS).

Bench Top Instrument

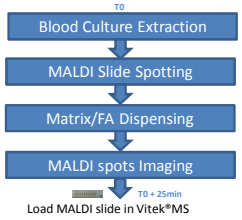

Figure 3 : Blood Culture Prep station prototype with the description of the different sub-systems : a) prepared rack b) pipetting tips and FWs plate holder c) MALDI slide holder including FA/CHCA reagents, 96 tips rack and waste d) ADP Tecan for FA and CHCA matrix dispensing e) MALDI spot Imaging sub-system including illumination LED.

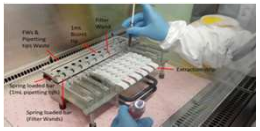

Figure 4. Run Preparation :

- Ready-to-use strip concept with embedded reagents
- 2min hand-on-time to load & fill strips with 1mL positive BC
- Load Vitek®MS target slide & CHCA, FA, *E.Coli* calibrant tubes
- Up to 12 BC could be extracted simultaneously by the instrument.
- Load Rack in the instrument and Start

Figure 5. Filter Wands Extraction & Spotting Steps. a) at the end of the positive BC vacuum aspiration, b) at the end of wash steps showing that all the aspirated liquids are contained in the FW body and blood component are moved far away from the filtration membrane to limit the pollution of MALDI slide with hemoglobin during the spotting step; c) After returning to atmospheric pressure, each FW is applied on each MALDI spot with a controlled strength

Fully Automated Sequence - 1 to 12 Blood Cultures per run. TOTAL TIME = 25 min

1. **Pick up Pipetting tip in rack. Aspirate Lysis buffer. Mix with BC. Discard tips to waste. Wait 2min at RT**
2. **Pick up Filter Wand in rack. Aspirate 2min in lysed BC**
3. **Move FW to different wash steps tubes under vacuum. (7 min duration)**
4. **Press successively all the FW on Vitek®MS spots to transfer microorganisms. Discard FW to waste**
5. **Dry spots 3 min at 45°C. Take target slide pictures**
6. **Dispense Formic acid, CHCA matrix and of *E.coli* calibrator**
7. **Dry spots 5-7min at 45°C. Take target slide pictures**
8. **Recover ready Vitek MS MALDI slide. Discard extraction strips to waste.**

Conclusions

Feasibility of a fully automated Vitek®MS target slide preparation from positive Blood cultures has been demonstrated on both seeded and clinical BC bottles. When associated to Vitek®MS, the prototype allowed to process up to 12 positive BC with less than 5mn hand-on-time and provided accurate ID within 1 hour.

Results

Correct Identification Rate (%) – Seeded Bottles			
Type of monomicrobial Microorganims	**Reference** (24-48h Sub-culture + Vitek®MS)	**BacT/Alert 3D**, SA Bottles (BC Prep Station + Vitek®MS)	**BacT/Alert Virtuo**, FA Bottles BC Prep Station + Vitek®MS)
Gram-negative species	**96 %**	**85%**	**86%**
Gram-positive species	**98 %**	**88%**	**86%**
Yeast species	**100 %**	**100%**	**75%**
Overall ID Rate	**97.3%**	**87%**	**84%**

Table 1. Global distribution of Vitek®MS identification results with rapid automated method using the BC Prep Station from seeded bottles incubated in BacT/Alert 3D® and BacT/Alert Virtuo systems versus reference subculture technique

HCL Hospital Standard Procedure (24-48h)			« BC Prep Station » + VitekMS (1h)		
Identified Microorganisms	Isolate		Tests	Correct ID	
	Number	Percentage	Number	Number	Percentage
Gram negative bacilli	**33**	**36%**	**66**	**59**	**89%**
Escherichia coli	13	14%	26	26	100%
Proteus mirabilis	4	4%	8	8	100%
Ent.cloacae/asburiae	3	3%	6	4	67%
Klebsiella pneumoniae	3	3%	6	6	100%
Acinetobacter baumannii	2	2%	4	4	100%
Klebsiella oxytoca	2	2%	4	4	100%
Pseudomonas aeruginosa	2	2%	4	3	75%
Fusobacterium nucleatum	1	1%	2	0	0%
Moraxella osloensis	1	1%	2	2	100%
Ochrobactrum anthropi	1	1%	2	2	100%
Pseudomonas putida	1	1%	2	0	0%
Gram positive bacilli	**1**	**1%**	**2**	**0**	**0%**
Propionibacterium acnes	1	1%	2	0	0%
Gram positive cocci	**49**	**53%**	**98**	**79**	**81%**
Staphylococcus epidermidis	22	24%	44	37	84%
Staphylococcus aureus	7	8%	14	13	93%
Enterococcus faecalis	4	4%	8	7	88%
Staphylococcus capitis	3	3%	6	5	83%
Micrococcus luteus/lylae	2	2%	4	2	50%
Staphylococcus warneri	2	2%	4	3	75%
Streptococcus constellatus	2	2%	4	1	25%
Staphylococcus haemolyticus	1	1%	2	2	100%
Staphylococcus hominis	1	1%	2	1	50%
Streptococcus agalactiae	1	1%	2	2	100%
Streptococcus dysgalactiae	1	1%	2	2	100%
Streptococcus gallolyticus	1	1%	2	0	0%
Streptococcus mitis/oralis	1	1%	2	2	100%
Streptococcus pneumoniae	1	1%	2	2	100%
Yeast	**9**	**10%**	**18**	**14**	**78%**
Candida albicans	3	3%	6	5	83%
Candida krusei	1	1%	2	2	100%
Candida lusitaniae	2	2%	4	3	75%
Candida parapsilosis	1	1%	2	2	100%
Candida tropicalis	1	1%	2	0	0%
Candida utilis	1	1%	2	2	100%
TOTAL	**92**		**184**	**152**	**83%**

Table 2. Direct identification vs standard procedure from clinical BC. Each Clinical BC were tested with 2 extractions duplicate and 2 spots per Filter Wand, leading to 4 Identification results per isolate.

Correct Identification rate were 89% for Gram-negative bacteria (33 isolates) , 78% Gram-positive (50 isolates) and 78% for Yeast (9 isolates).

Only 4 discordant cases :
P.aeruginosa identified as *P.aeruginosa* and *B.megaterium* ;
S.epidermidis identified as *S. hominis* ;
F.nucleatum identified as *V.parahaemolyticus and L.monocytogenes* ;
C.tropicalis identified as *C.albicans*

Reference Identification Mixed BC	BC Prep Station + Vitek®MS
Psychrobacter / S.epidermidis	*Psychro. phenylpyr*
E.faecalis / A.baumannii	*E. faecalis*
B.fragilis / S.hominis	*B. fragilis*
S.hominis / S.epidermidis	*S.hominis/S.epidermidis*
E.coli / C.glabrata	*E.coli*

Table 3. Polymicrobial results. On 5 confirmed polymicrobial clinical BC, the rapid automated method identified at least one of the pathogen present.