SP21

Optimizing Time to Identifying Growth From Short Incubation Cultures using WASPLab™ (Copan, Italy)

Laboratory Medicine & Pathobiology, University of Toronto

Sinai Health System

Kevin R Barker[1,2], Melissa Kissoon[1], Michael McConnell[1], Barbara M Willey[1], Pauline Lo[1], Tony Mazzulli[1,2], Susan M Poutanen[1,2]

[1] Sinai Health System/University Health Network, Toronto, ON, Canada. [2] Department of Laboratory Medicine and Pathobiology, University of Toronto, ON, Canada.

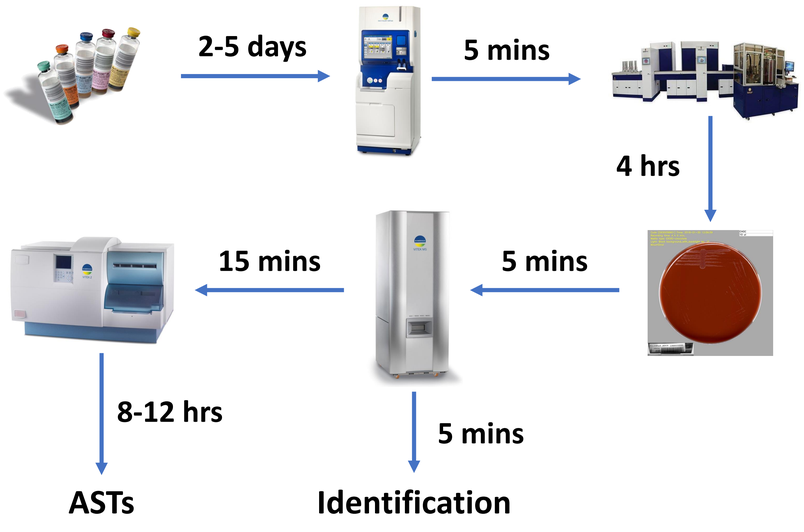
Kevin.barker@mail.utoronto.ca

INTRODUCTION

- Mortality due to sepsis is high with delays in diagnosis and delays in initiation of appropriate Rx linked to poor outcomes
- MALDI-TOF MS is useful to identify blood culture (BC) short-incubation culture isolates thereby improving identification turn-around-times (TAT) to <12 hours for most isolates
- WASPLab™ encompasses an integrated model of automated processing and smart incubation involving high-resolution imaging
- WASPLab™ combined with VITEK® MS (bioMérieux) has the potential to further improve BC ID TAT

WASPLab™

HYPOTHESIS



WASPLab™ combined with VITEK® MS allows rapid ID TAT of blood culture positivity

RESEARCH QUESTIONS

1) At what concentration do BC flag positive from the BACT/ALERT® VIRTUO® (bioMérieux)?

2) Using BC spiked with isolates at concentrations simulating flagged positive BC, how early can we detect growth and obtain ID using WASPLab™ imaging and VITEK® MS?

3) What are the optimal programmed WASPLab™ imaging times for BC subcultures in order to minimize TAT to ID?

4) Can we successfully ID BC short incubation cultures prospectively using WASPLab™ at these times?

RESULTS

Median prospective blood cultures flag positive at $4x10^{10}$ cfu/l (GPC) and $7x10^{11}$ cfu/l (GNB)

APPROACH: 52 prospective BC bottles <3 hrs of flag positive on the BACT/ALERT® VIRTUO® were serially diluted, and colony counts undertaken using the spread plate technique

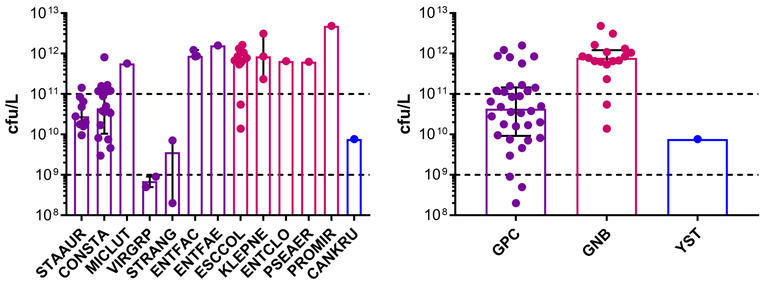


Figure 1.0. Prospective BC colony forming units per litre (cfu/L) at time of positivity. Median cfu/L of blood culture isolates at the time of positivity incubated in the BACT/ALERT® VIRTUO® blood culture system. (A) All BC (n=1-16/grp). (B) Grouped by Gram-positive cocci (GPC; n=34/grp); Gram-negative bacilli (GNB; n=17/grp) and yeast (YST; n=1). Circles represent individual values, error bars represent interquartile range. Dotted line indicates the concentrations chosen for retrospective blood cultures. Abbreviations: *S. aureus* (STAAUR); coagulase negative staphylococci (CONSTA); *M. luteus* (MICLUT); viridans-group streptococci (VIRGRP); *S. anginosus* (STRANG); *E. faecium* (ENTFAC); *E. faecalis* (ENTFAE); *E. coli* (ESCCOL); *K. pneumoniae* (KLEPNE); *E. cloacae* complex (ENTCLO); *P. aeruginosa* (PSEAER); *P. mirabilis* (PROMIR); *C. krusei* (CANKRU).

Retrospective blood cultures can be successfully identified as early as after 4 hrs of incubation

APPROACH: 68 previously frozen clinical isolates reflecting the most frequent and significant BC bacteria were processed by WASPLab™ at 10^{11} and 10^{9} cfu/L reflecting BACTI/ALERT® VIRTUO® flagged BC concentrations. HD digital images were taken at incubation intervals (0-48 hrs). MALDI-TOF MS was undertaken upon visible growth.

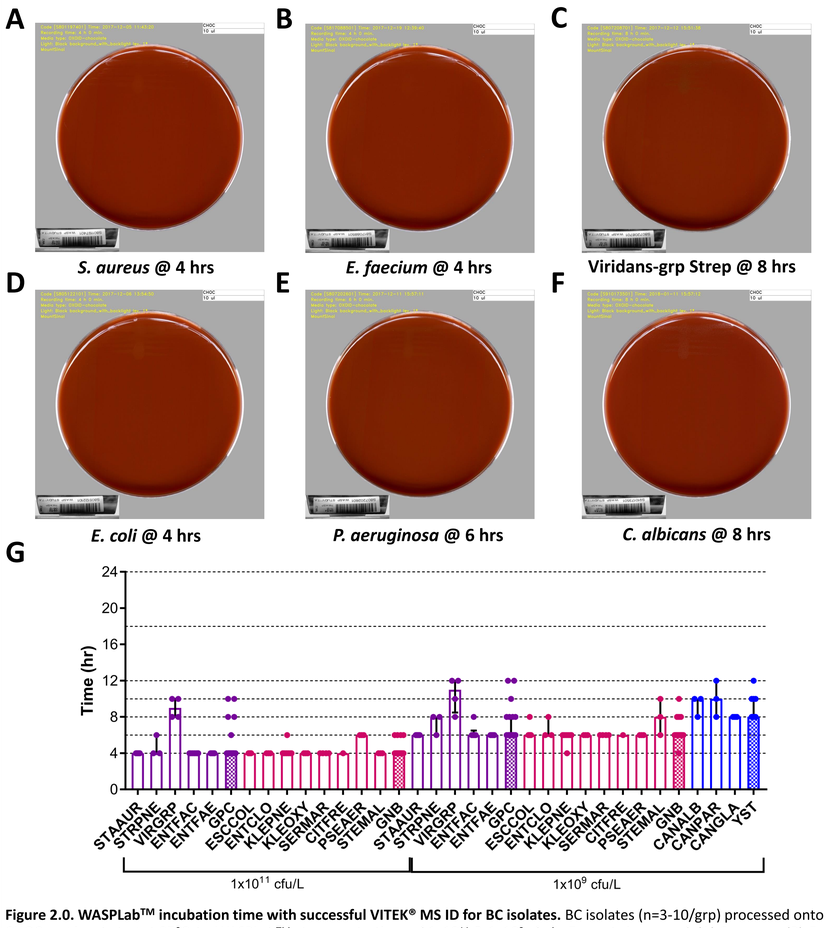


Figure 2.0. WASPLab™ incubation time with successful VITEK® MS ID for BC isolates. BC isolates (n=3-10/grp) processed onto CHOC and incubated at 37°C by WASPLab™ at concentrations of $1x10^{11}$ & $1x10^{9}$ cfu/L. Example images of: (A) *S. aureus*, (B) *E faecium*, (C) viridans-group streptococci (D) *E. coli*, (E) *P. aeruginosa*, at $1x10^{11}$ cfu/L; and (F) *C. albicans* at $1x10^{9}$ cfu/L. (G) Median time of incubation for successful VITEK® MS identification of ≥99.0% for all blood cultures (n=1-10/grp) and grouped GPCs (n=25/grp), GNBs (n=33/grp) and YST (n=9/grp). Circles represent individual values, error bars illustrate interquartile range. Abbreviations: *Streptococcus pneumoniae* (STRPNE); *Klebsiella oxytoca* (KLEOXY); *Serratia marcescens* (SERMAR); *Citrobacter freundii* (CITFRE); *Stenotrophomonas maltophilia* (STEMAL); *Candida albicans* (CANALB); *Candida parapsilosis* (CANPAR); *Candida glabrata* (CANGLA)

Recommended programmed WASPLab™ imaging times for BC subcultures to minimise ID TAT: 4, 10, 18, and 48 hrs.

Prospective blood cultures can be successfully identified as early as after 4 hrs of incubation

APPROACH: 30 prospective blood cultures bottles were processed by WASPLab™ within 5 hrs of flagging positive by BACTI/ALERT® VIRTUO®, and ID undertaken by VITEK® MS upon visible growth.

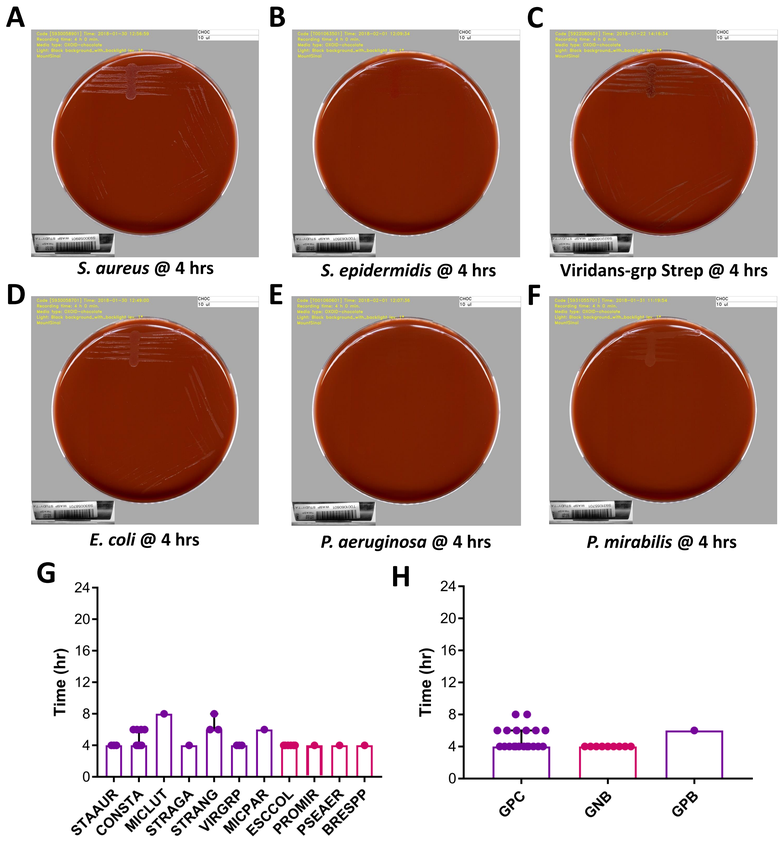


Figure 3.0. WASPLab™ incubation time with successful VITEK® MS identification for prospective blood cultures. Prospective blood culture isolates (n=1-8/grp) processed onto CHOC and incubated at 37°C with 5% CO_2 by WASPLab™. (A-F) Example images of (A) *S. aureus*, (B) *S. epidermidis*, (C) viridans-group streptococci, (D) *E. coli*, (E) *P. aeruginosa,* (F) *P. mirabilis* –positive colonies after 4 hrs incubation. (G-H) Median time of incubation for successful VITEK® MS ID of ≥99.0%. (G) All BC isolates (n=1-8/grp). (H) All BC grouped by GPC (n=20/grp), GNB (n=9/grp), and Gram-positive bacilli (GPB; n=1/grp). Circles represent individual values, error bars illustrate interquartile range. Abbreviations: *S. agalactiae* (STRANG); *S. anginosus* (STRANG); *Microbacterium paraoxydans* (MICPAR); *Brevibacillus* spp (BRESPP)

SUMMARY

1) BC flag positive at **$4x10^{10}$** (GPC) & **$7x10^{11}$** (GNR) cfu/L

2) Median time (hr) to ID at **$1x10^{11}$ cfu/l**:
GPC = 4 (IQR = 0); GNB = 4 (IQR = 0)
Median time (hr) to ID at **$1x10^{9}$ cfu/l**:
GPC = 6 (IQR = 2); GNB = 6 (IQR = 0); YST = 8 (IQR = 2)

3) Optimal imaging times = **4**, **10**, **18** and **48 hrs**

4) Median time (hr) to ID **prospectively**:
GPC = 4 (IQR = 2); GNB = 4 (IQR = 0)

WASPLab™ combined with VITEK® MS facilitates successful BC ID as early as after 4 hrs of incubation

FUTURE DIRECTIONS

- Verification & implementation of the WASPLAB™ PhenoMATRIX™ AI system to recognize & segregate positive short incubation growth images at 4, 10, 18, and 48 hrs
- Verification of AST from short incubation cultures