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An automated sample preparation instrument to accelerate positive blood culture microbial identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (VitekMS)

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Background: The diagnosis of bloodstream infections must be as fast as possible to ensure effective antimicrobial therapy. Thus, rapid methods are required on blood culture (BC) samples to reduce the time to obtain microorganism identification and to determine antibiotic sensitivities. Recently, many methods aiming to achieve direct identification on BC samples by MALDI-TOF MS have been described but they are still manual, need the availability of dedicated technician in routine practice and are based on batches workflow which impair the time saving. To address these issues we present a new bench-top prototype for microorganism fully automated extraction and MALDI-TOF MS plate preparation. We compare the performances, obtained directly from seeded and clinical positive BCs, with reference methods using subculture.

Material/methods: The instrument prototype (Figure 1) is based on a “all-in-one” extraction strip and specific filtering device, covering the missing links between bacT/Alert   and Vitek  MS systems (bioM  rieux). This instrument, starting from 1mL positive BC, performs BC extractions (selective lysis, microorganisms capture, washings) and finalizes the Vitek  MS slide preparation by microorganisms spotting, matrix dispensing, drying and target slide imaging. This prototype was first evaluated on 111 strains (60 species) seeded in SA/SN bottles, incubated in BacT/Alert   3D and on 22 strains seeded

in FA/FN Plus bottles, incubated in bacT/Alert Virtuo™ system. After optimization, the prototype was field tested on 102 clinical BC in a French clinical microbiology laboratory (HCL, Lyon). Four identification results were obtained for each samples processed with the prototype and compared to results obtained with standard workflow using subculture of positive blood bottles and identification by VITEK MS system.

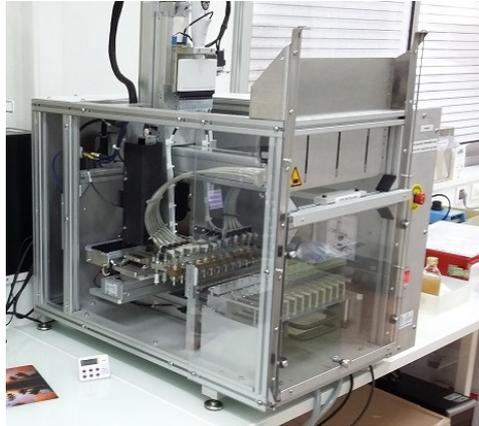


Figure 1: Bench-top prototype for fast BC extraction and MALDI slide preparation

Results: The overall performance achieved from spiked BC bottles was 87% of correct identification (386 correct identifications on 442 results; Gram-negative=85%, Gram-positive=88%, Yeast=100%) when used with bacT/Alert® 3D and 84% (74 correct identifications on 88 results; Gram-negative=86%, Gram-positive=86%, yeast=75%) with Virtuo™. For clinical BCs, the overall correct identification rate on 97 monomicrobial bottles was 82% (Gram-negative=89%, Gram-positive=78%, Yeast=78%). For the 5 confirmed polymicrobial bottles, 5 were correctly identified with 1 species on the 2 present in the positive bottles.

Conclusions: This small size instrument was able to process up to 12 blood cultures within 25 min, needed only 5 min hand-on-time preparation, didn't need daily decontamination and delivered a ready-to-use MALDI-TOF MS slide including formic acid and CHCA matrix dispensing. This proof of concept study demonstrated performances close to published manual extraction methods (~80% correct identification for Bruker's and bioMerieux's manual commercial kits). The main benefit of this instrument was to improve routine positive BCs workflow management, thank to fast, flexible and fully automated processing dedicated to direct positive blood culture microorganism rapid identification.