

Comparison of traditional blood culture and Hyborg Dx in patients suspicious for sepsis

Introduction

Sepsis is a potentially life-threatening complication of infection. Microbial organisms entering the blood stream lead to an inadequate inflammatory response with the risk of consecutive multi organ failure. A correct and timely diagnosis is therefore of the utmost importance. Blood culture (BC) is the current mainstay in the diagnosis of sepsis. It allows the isolation of viable microorganisms which can be analyzed to identify species and antimicrobial susceptibility. However, drawbacks of BC include the delay in the time from sampling to results and the unsatisfactory sensitivity. BC is only positive in approximately 30% of these patients.

Materials and Methods

Samples from 83 in-patients of the intensive care unit suspected for sepsis were analyzed by traditional blood culture using the BACTEC™ 9240 (Becton Dickinson GmbH, Germany) blood culture system during routine purposes. In all cases corresponding EDTA-blood was analyzed with the hyborg Dx (Cube Dx, Austria). The hyborg Dx is a fully integrated and automated microarray based analysis system for DNA and protein assays using hybcell technology. DNA extraction was conducted with the Arrow System™ (Molzym, Germany) using MolYsis™ (Select NA) - Pathogen DNA Isolation Kits (Molzym, Germany). The detection limit of the Hyborg Dx was determined with serially diluted pure cultures of *Staphylococcus aureus* and *Candida albicans* with known concentrations of colony forming units per milliliter (CFU/ml).

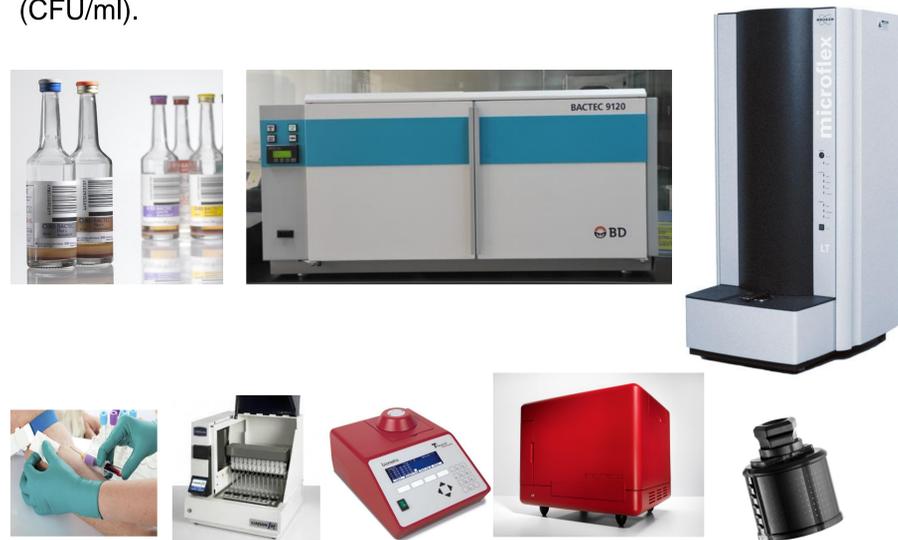


Figure 1: Traditional blood culture workflow using BacTec and microflex LT in comparison to molecular based Cube Dx workflow using EDTA-blood, DNA extraction, amplification using endpoint PCR and hybcell detection

Results

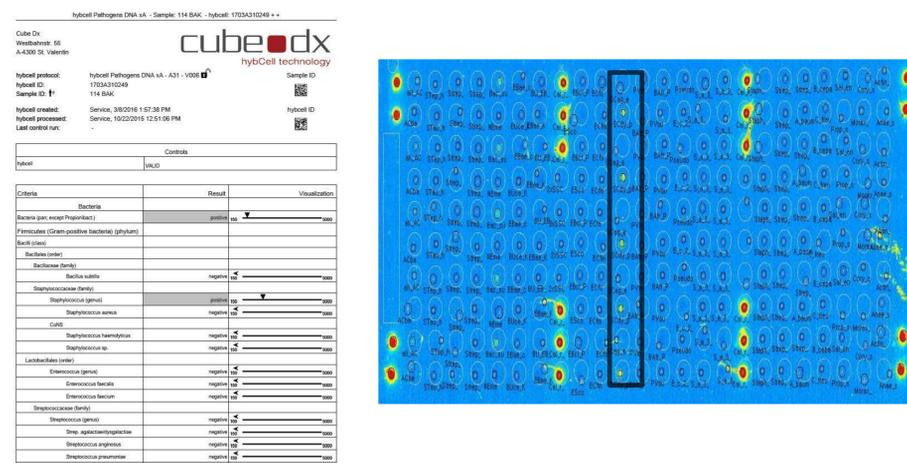


Figure 2: Exemplary Cube Dx result report (left) and corresponding hybcell surface with a positive result (black rectangle) for *Streptococcus* and red control and position spots

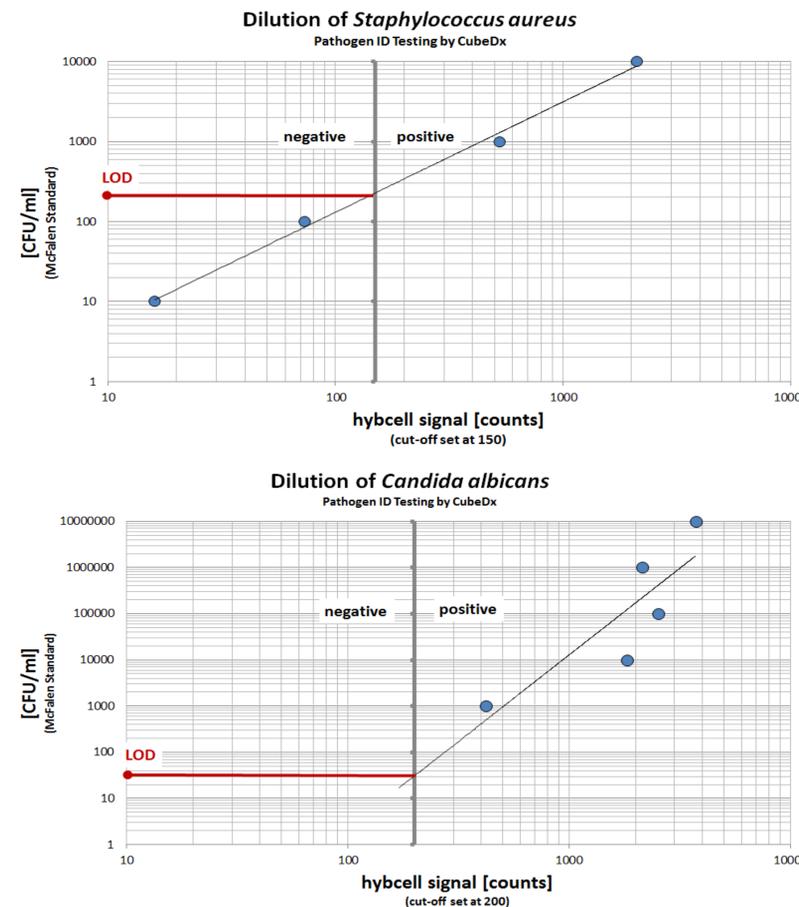


Figure 3: Dilution series (1.00E+00 – 1.00E+06 CFU/ml) of *Staphylococcus aureus* and *Candida albicans*. The limit of detection (LOD) is marked as vertical grey line.

Results

	Hyborg Dx positive	Hyborg Dx negative	Overall concordance	Sensitivity	Specificity	NPV	PPV
Blood culture positive	13	5	71.1%	72%	71%	90%	41%
Blood culture negative	19	46					

Table 1: Statistical evaluation of the correlation between traditional blood culture and the Hyborg Dx (NPV: negative predictive values; PPV: positive predictive value)

Of 83 samples 18 (21.7%) showed growth in conventional blood cultures with a median time to positivity of 9.5h (min. 7.5h to max. 25h). With the hyborg Dx microorganisms were detected in 32/83 samples (38.6%). 55.4% of the samples (46/83) were classified as negative by both test methods. 15.7% (13/83) were classified as positive by BC and the hyborg Dx. In 11 of these 13 cases (84.6%) both methods detected the same species. With BC as the gold standard 5/83 (6%) of the hyborg Dx results were classified as false negative and 19/83 (22.9%) as false positive, leading to a sensitivity of 72% and a specificity of 71% with a negative predictive value (segregant; NPV) of 90% and a positive predictive value (PPV) of 41%. 13/19 (68.4%) were under antibiotic treatment before or during sampling. In 6/19 (31.6%) no information was available. Analyzing dilution series using the hyborg the lowest detectable concentration of *Staphylococcus aureus* and *Candida albicans* was found to be 1.00E+03 CFU/ml for both pathogens. The detection limit of the BC is published as 1.00E+02 CFU/ml.

Conclusions

The hyborg Dx might be an alternative to other commercially available molecular based test systems. It provides short hands on time and a short turnaround time of 5h. The detection limit is comparable to the BC. The adaption of the cut off to reduce potential false positive test results and the omission of sequence homologies of the used oligonucleotides to avoid wrong genotyping of the pathogens is required for routine use.

References

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2. Parikh K, Davis AB, Pavulun P: Do we need this blood culture? ;Hosp Pediatr. 2014 Mar;4(2):78-84. doi: 10.1542/hpeds.2013-0053..
3. O. Liesenfeld, L. Lehmann, K.-P. Hunfeld, G. Kost: Molecular diagnosis of sepsis: New aspects and recent developments; Eur J Microbiol Immunol (Bp). 2014 Mar; 4(1): 1–25.