Assessment of a light-scattering system in the culture screening of biological fluid samples

Summary
The diagnosis of invasive infections is commonly based on the cultures of pathogenic microorganisms; the morphological and biochemical identification is followed by several types of antimicrobial susceptibility tests. Early identification and rapid antimicrobial susceptibility testing of microorganisms, particularly in the case of serious infections, represent high priorities in clinical microbiology. Our study aimed at evaluating a semiautomated system (HB&L by Alifax S.p.A., Padova, Italy) for the recovery of microorganisms from sterile body fluids other than traditional cultural methods.
HB&L offers the possibility for the culture of an additional 5.8% samples, if compared with the reference cultures. The two methods showed good concordance (85%), with high sensitivity specificity (97.87%) and positive predictive value (98.43%%) and very good and negative predictive value (86.3% and 82.14%, respectively). The microbial counts showed excellent agreement in the results between HB&L and traditional methods.

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Introduction

Quality assurance in health care is a concept that is rapidly gaining importance all over the world; a positive culture and sensitivity reports help clinicians to confirm a diagnosis as well as to modify the initial therapy[1,2]. In the case of serious infections, a timely report can prove life saving, and a rapid detection of the causative pathogens is important for a prompt and an appropriate antimicrobial treatment[1,2]. However, negative reports are also important as they enable physicians to stop any empirical antibiotics that might have been started earlier. By the conventional methods for culture and sensitivity testing employed by the laboratories, it would be difficult to shorten turn-around times significantly[1,2]. The necessity to obtain reliable and quick results has arisen from the spread of automated diagnostic methods in microbiology[4,12]. In the last years a semiautomated system, HB&L (Alifax S.p.A., Padova, Italy), which uses light scattering technology to detect the growth of bacteria, has been employed for bacterial screening in urine and several biological samples and it has offered rapid outcomes[4,11].

Materials and methods

120 varied body fluids submitted for culture to the microbiological laboratory of the hospital of Trento were included in the study. All sample culture sets were processed for the purpose of patient care and processed within 2 h after the collection[6,9]. A minimum volume of 1 ml was required for the specimen to be included in the study. The specimen types included in the study (table 1) were 22 pleural, 8 peritoneal, 18 ascitic, 10 synovial,

8 cerebrospinal (CSFs),1 pericardial fluids and 53 lower respiratory tract samples (LRSs) distinguished in 8 bronchoalveolar-lavage, 26 sputum, 12 endotracheal and 7 broncho-aspirates. According to the Murray-Washington scheme, only LRSs displaying a microscopic examination score of 5 were included. Viscous sputum were fluidized with Sputasol (Oxoid, Basingstoke, UK) and the final volume was considered for the correct microbial counting[3,10]. When specimens arrived in the laboratory, 0.5 ml of the fluid sample was used for the HB&L screening and the remaining volume was cultured by conventional methods. Samples were centrifuged at 1180 rpm for 30 min at room temperature and the pellet was inoculated into conventional solid media: CO2-supplemented chocolate agar, 5% sheep blood agar incubated aerobically or anaerobically (the latter to enhance the recovery of some streptococci), MacConkey agar, and Saboraud agar. Microbial enumeration was conducted on each fluid sample before centrifugation following a conventional procedure. Samples inoculated on solid media were incubated at 37°C for 24 h. If no growth was evident, incubation was continued for five days[6,9]. All the microorganisms isolated from the samples were identified using the MICROSCAN® WA 96 (Siemens Healthcare Diagnostics).

The HB&L system is based on a light-scattering technique that reliably detects microbial growth in fluid samples, providing real-time growth curves and bacterial counts (cfu/ml). The system was originally designed for the rapid screening of urine samples. However, the basis of the detection (nephelometric detection of light scattering) potentially makes the system widely applicable[4,11]. The sensitivity of the system, expressed in terms of colony forming units/millilitre (cfu/mL), depends on the time of detection: the time required to detect 100,000 cfu/mL is 180 minutes. The incubation must be prolonged to 235 min if the detection cut-off is lower than 1000 cfu/ml and 6 h to have a cut-off <50 cfu/ml. The HB&L supports the growth of diverse bacteria in combination too, including those that are clinically relevant and the microrganisms producing the small colony variant[4].

Upon arrival in the laboratory, 500 µl of each fluid sample were dispensed in the culture. Considering the necessity of culturing fastidious microorganisms such as *Neisseriae* and *Haemophilus* in CSF, each vial was supplemented with 200 µl of a commercial preparation (Alifax) containing nicotinamide-adenine-dinucleotide, factor X, and hemin[5,7]. The fluid samples were cultured in the HB&L for 360 min to achieve the cut-off <50 cfu/ml. At that time the microbial counts were obtained, and aliquots of the culture from the culture vials were dispensed onto the medium used for the traditional culture[4,6,11]. The results of the conventional culture

Table 1. Samples and positive results of HB&L system and reference cultures.

Samples	n° of samples	n° positive by culture (%)	n° positive by HB&L (%)	n° positive only by culture (%)	n° positive only by HB&L (%)
Sputum	26	17	22	1	3
Endotracheal-aspirates	12	9	10	-	-
Broncho-aspirates	7	2	3	-	-
Bronchoalveolar-lavage	8	2	3	-	1
Pleural fluids	22	1	4	-	1
Peritoneal fluids	8	4	5	-	1
Ascitic fluids	18	4	6	-	1
Pericardial fluids	1	-	-	-	-
Synovial fluids	10	1	3	-	-
Cerebrospinal fluids	8	-	-	-	-
TOTAL	**120**	**40 (33.3%)**	**56 (46.6%)**	**1 (0.8%)**	**7 (5.8%)**

Table 2. Results of HB&L system.

Samples	True pos. HB&L (%)	True neg. HB&L (%)	False pos. HB&L (%)	False neg. HB&L (%)	Clinically relevant microrganisms isolated (n°)
Sputum	18	3	4	1	*Staphylococcus aureus(4), Haemophilus influenzae(2), Pseudomonas aeruginosa(3), Acinetobacter lwoffi(1), Enterobacter cloacae(1), Candida albican(1), Aspergillus fumigatus(1)*
Endotracheal-aspirates	9	2	1	-	*Pseudomonas aeruginosa(6), Candida glabrata(1)*
Broncho-aspirates	3	4	-	-	*Staphylococcus aureus(1), Pseudomonas aeruginosa(1)*
Bronchoalveolar-lavage	3	5	-	-	*Staphylococcus aureus(1), Aspergillus fumigatus(1)*
Pleural fluids	2	18	2	-	*Escherichia coli(1), Streptococcus pneumoniae(1)*
Peritoneal fluids	5	3	-	-	*Staphylococcus aureus(1), Pseudomonas aeruginosa(1), Enterobacter aerogenes(1)*
Ascitic fluids	5	12	1	-	*Staphylococcus aureus(1), Pseudomonas aeruginosa(1), Escherichia coli(1), Enterobacter cloacae(1)*
Pericardial fluids	-	1	-	-	-
Synovial fluids	1	7	2	-	*Staphylococcus aureus(1)*
Cerebrospinal fluids	-	8	-	-	-
TOTAL	**46 (38.3%)**	**63 (52.5%)**	**10 (8.3%)**	**1 (0.8%)**	

were compared with those obtained using HB&L.

In order to evaluate the ability of the HB&L system to support the growth of fastidious bacteria such as *Neisseria* spp. and *Haemophilus* spp, standard reference strains were used: *Neisseria lactamica* ATCC 23970, *N. sicca* ATCC 9913, and *Haemophilus influenzae* ATCC 49247.

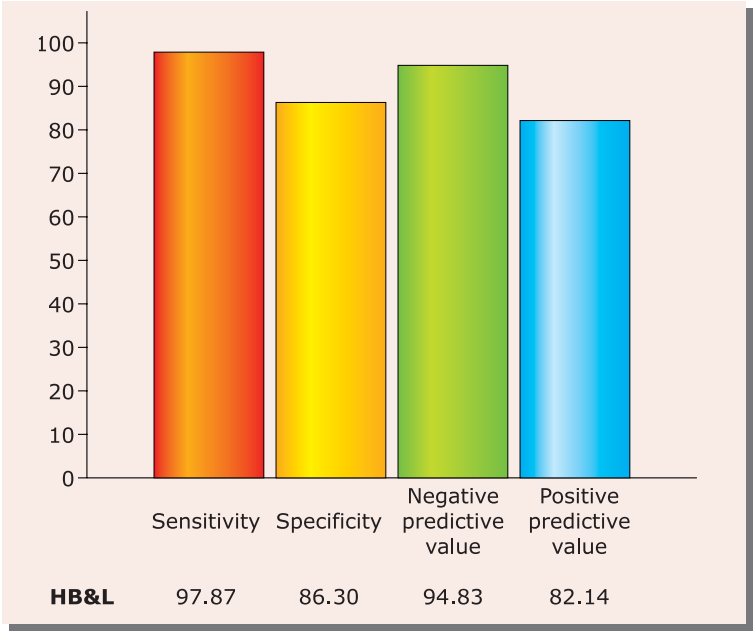
Results

Out of the 120 sterile body fluids samples analyzed 56 (46.6%) were positive with HB&L, while clinically significant bacteria were isolated from 32 (26.6%) by the reference method. Gram negatives bacteria were isolated from 34 (59.4%) samples, *Pseudomonas aeruginosa* was the most represented species. 14 (25%) samples were positive for Gram positives bacteria, in particular *Staphylococcus aureus*; fungi were isolated (2 *Aspergillus fumigatus,* 2 *Candida spp.*) in 4 samples. No anaerobic bacteria were isolated and in 12 specimens the microrganisms were of more than 1 species. Comparing the reference culture method and the HB&L system, total agreement was reached for 102 samples (85% of total specimens): 53.3% were true negatives and 0.8% false negatives. In the relevant mismatches between the two methods, verified on only a false negative sputum, the microorganism was *Staphylococcus aureus* and the patient was in therapy with glycopeptides.

Growth was detected only by the HB&L in 7 additional specimens (5.8%) and 8 (6.6%) LRSs positive with HB&L were considered contaminated with heavy resi-

dent bacteria by cultures. So 38.3% specimen were true positive and 8.1% were false positive.
Among the 7 specimens in which growth was exclusively and significantly detected by HB&L were comprised sputum, bronchoalveolar lavage, peritoneal, ascitic and pleural fluid.
For 22 samples HB&L pointed out a note of high turbidity but traditional cultures did not highlight any such discordance in these specimens.
Sensitivity (97.87%) and negative predictive value (98.43%) was excellent. Also the resulting specificity and positive predictive value was very high: 86.3% and 82.14% respectively.
Microbial counts obtained using HB&L were comparable with conventional results.

Figure 1. Qualitative performances of HB&L system.



Conclusions

Managed care, lab consolidations, and hospital mergers are forcing hospital labs to become cost-effective without sacrificing quality. It has become obvious that the prompt release of lab results can help reduce hospital costs under DRG/PPS arrangements. The clinical laboratory can implement cost savings by improving the physicians' utilization of the lab and by modifying test procedures.
Laboratories today are becoming increasingly short staffed with a severe skilled-labor issue in the microbiology domain. Labs are working to become more efficient and implement options for streamlining operations and reducing costs, while coping with increasing workload volumes and the demand for faster results from clinicians. Shortening the turnaround time of microbiological procedures was associated with an improved clinical outcome and can have a significant impact on the management of infections. The role of lab automation has become very important[12].
In this context, the development of instrument-based methods for rapid detection and identification of microorganisms plays a crucial role. Among semi-automated system, HB&L represents a new tool for a rapid screening of the presence of microorganisms in sterile body fluids specimens with the employment of an enrichment broth.
The results prove the validity of HB&L system compared with the traditional culture-based approach in the recovery of bacteria, including fastidious ones, in biological fluid samples. The additional 5.8% of positive samples is evidence of a light scattering technique's superiority over culture methods. Also the microbial count using HB&L system was accurate, easy and less labor intensive than conventional methods.
The system is easy to use and allows clinicians to discriminate positive samples from negatives ones in a very short time (maximum 6 hours) with an high level of reliability[4,8,11]. HB&L system represents an efficient instrument for a prompt diagnosis and treatment of infections[4]. TiM

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