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A New Method For Microbiological Analysis That Could Be Used For Point-Of-Care Testing (POCT)

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Abstract: Standardized microbiological methods used in clinical analysis are based on traditional microbial enrichment on selective media, possibly followed by characterization of bacteria with molecular methods. These techniques present several difficulties, such as the subjectivity in the interpretation of genetic, biochemical or morphological tests and the possible interference of biological matrices, specially when low levels of contamination are present. In addition, standardized microbiological analyses are characterised by the high cost of the method, both in terms of labor and supplies, and above all, by the long time needed to obtain definitive results (from 3 to 7 days). These reasons have led to the development and refinement of microbiological POCTs which are now available for several microorganisms, even thought no microbiological POCT was up to now developed for the count of total viable bacteria (TVC) in serum, urine or other biological fluids.

MBS srl (a spin-off of Roma Tre University, Rome, Italy) has developed and patented an alternative method for selective counting of bacteria, called Micro Biological Survey (MBS) method. The MBS method is based on colorimetric survey performed in mono-use disposable reaction vials in which samples can be inoculated without any preliminary treatment. The analyses can be carried out by untrained personnel and anywhere they are necessary, without the need for any other instrumentation than a thermostated optical reader that can automatically detect the colour change providing the number of bacteria present into the sample. The MBS method measures the catalytic activity of redox enzymes in the main metabolic pathways of bacteria, allowing an unequivocal correlation between the observed enzymatic activity and the number of viable cells present in the samples. The time required for a color change is inversely related to the log of bacterial concentration; like an enzymatic reaction, the greater the number of bacteria, the faster the color change.

The objective of this study was the primary validation, in accord with ISO 13843:2003 (Guidance on validation of microbiological methods), of the quantitative Micro Biological Survey (MBS) method for Total Viable Count (TVC). Validation aims to compare the results obtained with an alternative method, in this case the MBS method, with the results obtained with the reference method. To verify the equivalence between the two methods different parameters were analyzed: selectivity, linearity and accuracy. The validation has shown that the MBS method gives similar results and is in agreement with the reference methods. The MBS method could therefore represent a worthy aid in microbiological analysis as POCT device without replacing the analysis carried out with traditional methods which are very precise though often long and complex.

Keywords: Urinary Tract Infections (UTI), Antibiotic therapy, Point-of-care testing (POCT), Microbiological analysis, Antibiogram, Primary validation, Micro Biological Survey (MBS) method.

INTRODUCTION

Standard microbiological methods used in clinical analysis are based on traditional microbial enrichments on selective media, followed by characterization of bacteria with phenotypic methods. Although effective, these techniques present several problems, such as the subjective interpretation of metabolic, biochemical or morphological tests and the possible interference of biological matrices, especially when microbial contamination is low. In addition, standardized microbiological analyses are characterised by significant cost, both in terms of labour and supplies, and above all, by the long time (up to several days) needed to obtain definitive results. These reasons have led to the development and refinement of microbiological point-of-care tests (POCTs) which are now available for specific microorganisms present in blood, urine or cerebral spinal fluid [1]. However, particularly for urinary tract infections (UTIs), there are no microbiological POCTs developed for the rapid assessment of total viable bacteria.

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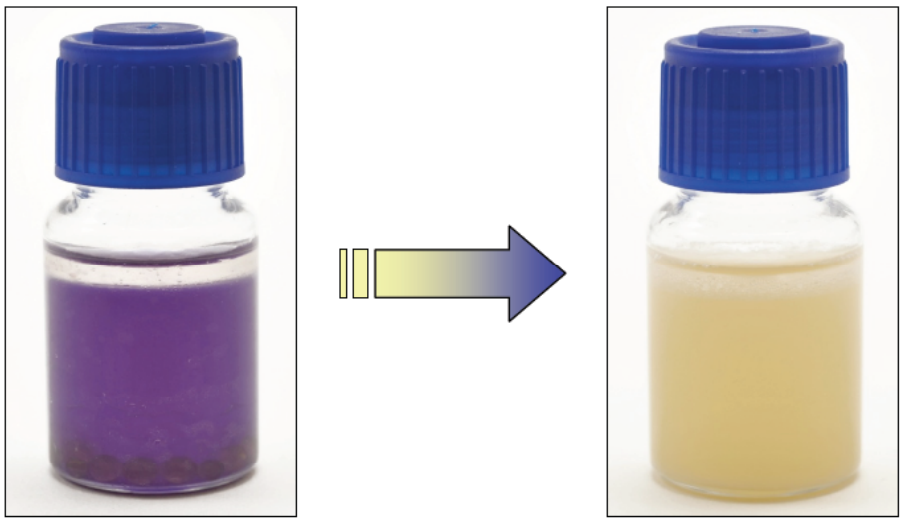

Fig. (1). Colour change of the MBS vials
The colour change of the mono-use disposable reaction vial (provided with sterile MBS original reagents) occurs in the presence of bacteria. The starting colour blue (left), changes to yellow (right) in the presence of bacteria. In the absence of bacteria the colour remains blue. The time required for colour change is inversely related to bacterial concentration, almost independently on the bacterial strains present into the vial, on the only condition that the bacteria present into the vial are able to consume oxygen.

Roma Tre University (Rome, Italy) has developed an alternative method for the selective assessment of the bacterial load in diverse samples, called Micro Biological Survey (MBS) method [2]. The MBS method is based on a colorimetric survey performed in single-use disposable reaction vials (see (Fig. **1**)) in which samples can be inoculated without any preliminary treatment. The MBS method measures the catalytic activity of redox enzymes in the main metabolic pathways of bacteria, allowing an unequivocal correlation between the observed enzymatic activity and the number of viable cells present in the samples. Aerobic (including facultative anaerobic) bacteria can be detected and counted by the MBS method since the time required for a colour change is inversely related to the logarithm of the bacterial concentration. Like an enzymatic reaction, the greater the number of bacteria, the faster the colour change [3].

We have carried out a preliminary validation study, in accordance with ISO Standards, of the quantitative MBS method to explore the possibility of its use as microbiological POCT in UTIs, since the number of bacterial species involved in UTIs is relatively limited and nearly all of them are endowed with an aerobic metabolism. The present validation study has been focused on the bacteria more frequently responsible for UTIs and the obtained results have encouraged us to continue the study.

MATERIALS AND METHODS

- Bacterial species and strains tested were: *Escherichia coli* (ATCC 25992), *Enterobacter cloacae* (ATCC 13047), *Enterococcus. faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 12600), *Staphylococcus epidermidis* (ATCC 12228).
- Bacteria inocula were in the range 10^0-10^8 Colony Forming Units/ml (CFU/ml) and were prepared by diluting bacteria either in PBS or in urine from normal individuals.
- MBS Vials' shelf-life is more than 6 months at room temperature while the experiments lasted about 2 months.
- The colorimetric MBS method was used according to the previously described procedure [4, 5].
- The standard plate dilution method with enumeration of colony-forming units (CFU) on Trypticase Soy Agar (TSA) after 24-48 h incubation at 37°C was used as the reference method for the assessment of the bacterial concentration in biological samples [4].
- Data analysis was carried out according to ISO 13843:2003 [6] and ISO 16140:2003 [7] comparing the results obtained with the MBS method with the results obtained with the reference method (colony count on PCA) by looking at the following statistical parameters: Precision (one-way and two-ways analysis of variance, ANOVA); Coefficient of Variance (CV); Uncertainty (c^2 analysis); Linearity (correlation plots of the bacterial concentrations (10^0-10^8 CFU/ml) with the time required for the colour change in MBS vials); Accuracy (correlation plots of the CFU log obtained using the reference method with those obtained using the MBS method).

RESULTS

Precision, variance, uncertainty, linearity and accuracy of the MBS method were determined for all the bacterial species. Notably, the results obtained were almost independent from the bacterial species/strain utilised and no difference was observed between bacteria suspended in PBS or in urine from normal individuals.

- PRECISION: One-way analysis of variance: F=1.4 (Degree of Freedom (DF): 1,30 limit 1%= 4.17); Two-ways analysis of variance: F=2.0 (DF: 7,28 limit 1%= 2.27). Thus, no statistically significant differences between the results obtained with the reference

method and those obtained by the MBS method were observed.

- COEFFICIENT OF VARIANCE (CV). Reference method CV=0.2; MBS method CV=0.06. The MBS method resulted more reliable than the reference method.
- UNCERTAINTY (DF: 9 limit 0.5 % = 1.735): Reference method $\chi^2 \leq 0.25$; MBS method $\chi^2 \leq 0.22$. Uncertainty of the bacterial counts was demonstrated to be less for MBS method than for the reference method.
- LINEARITY The time required for a colour change is inversely related to the bacterial concentration in linear correlation: the greater the number of bacteria, the faster the colour change (linear equation: log CFU/ml= 7.027 -0.32 * t (hours); R^2= 0.973). For 10^5 CFU/ml, colour changes occurred after ca. 5 hours.
- ACCURACY: A slope close to 1.00 and an intercept close to 0 were observed (linear equation y= 1.01 * x -0.05; R^2= 0.9526), indicating the almost complete agreement between the MBS method and the reference method.

CONCLUSIONS AND PERSPECTIVES

Preliminary validation tests here reported demonstrated that the MBS method provided results that were in line with the reference method of colony count on PCA for the bacterial species most commonly present UTIs. The MBS method could therefore represent a worthy aid in clinical microbiology as a POCT device to be used for UTI patients when the infecting bacterial species are reasonably predictable. The MBS method could also be used as enrichment medium before the conventional identification of the infecting bacterial species and the assessment of antibiotic susceptibility are performed. The research will continue analysing with the MBS method the urine from patients with and without a clinical and laboratory diagnosis of UTI.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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