

Sysmex UF-1000i flow cytometer capability to discriminate Gram negatives and Gram positives in urine

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Background. Rapid identification of bacterial pathogens in urine is essential to prescribe an adequate empirical antibiotic therapy to treat urinary tract infections (UTIs). UF-1000i, a flow cytometer for the evaluation of the particulate elements in urine, is capable of reproducible measurement of bacteria and it is now accepted and used in many microbiological laboratories for screening of UTI for its precision, automation, standardization and above all for the very short TAT for reporting results of negative urine samples.^{1,2}

In this study, as well as the reevaluation of the optimal cut-off for bacterial counts, we wanted to assess the instrumental ability to discriminate the type of causative organism in the positive screened samples using the technical parameter B_FSC of the bacteria channel with the aim to get directions on antibiotic therapy earlier as possible.

Methods. The UF-1000i (Sysmex Corp, Japan), has a dedicated analytical channel for precise bacteria detection and counting. In this channel the size and the nucleic acid content of bacteria are evaluated by means of two parameters, the B_FSC (Bacteria Forward Scatter) and the B_FLH (Bacteria Fluorescent Light Intensity) expressed in arbitrary units (ch) that provide informations about microorganism morphology.

1659 consecutive midstream urine specimens from in- and outpatients, representative of gender and of all age groups were examined by Sysmex UF-1000i and cultured on CHROMAgar Orientation and CNA agar plates (Kima, Padua, Italy); in culture positive samples bacterial identification was achieved by Vitek2 system (bioMerieux, Florence, Italy). Statistical analysis was performed by ROC curve analysis for obtaining the optimal cut-off for bacterial and leucocyte count. The data from the Gram negative and Gram positive bacteria were evaluated and the distribution of B_FSC values in the two groups was compared by the Mann-Whitney test.

Results. 1144 samples were negative at culture (68,9%). Out of 515 bacterial culture positives (31,1%), 345 samples presented a growth of Gram negatives, 61 a growth of Gram positives, in 24 samples a Gram positive and a Gram negative were identified, in 6 samples *Candida* spp was identified and 79 samples showed mixed growth (contamination). At a cut-off for bacterial count of 100 bacteria/ μ L and 150 leucocytes/ μ L, sensitivity was of 98,9%, specificity was 77,8% and VPN 99,4% (ROC curve analysis).

The median B_FSC was 20.05 ch. in Gram negatives and 48.1 ch. in Gram positives ($p < 0.0001$). Out of 294 samples with a B_FSC value < 30 ch., 256 (87.4%) were from samples with Gram negatives, 7 (2.4%) from Gram positives and 31 (10,5%) from mixed growths. 250 samples showed a B_FSC value < 25 ch.; 224 (89,6%) were Gram negatives, 2 (0,8%) Gram positives and 24 (9,6%) were mixed growths (Figure 1). B_FSC at a cut-off value of 30 ch. showed a sensitivity of 72.2% and a specificity of 80.8% in predicting Gram negative staining and at a cut-off of 25 ch. specificity raised at 87.7% and a sensitivity of 63%.

Conclusion. In our evaluation UF-1000i has confirmed a very good performance in a rule out strategy for negative samples. In the positive samples the instrument provide very useful informations about bacterial morphology. In fact when B_FSC value is < 25 ch in $\approx 90\%$ of cases a Gram negative occurs in culture while we have a very low probability (0,2%) to find a Gram positive.

B_FSC is a technical parameter henceforth proposed in the main page of instrumental scattergram with high specificity in recognizing Gram negatives in urine, if an optimal cut-off is selected, allowing to obtain in a few minutes informations about the aetiological agent for optimizing management of suspected UTI and guiding antibiotic treatment.

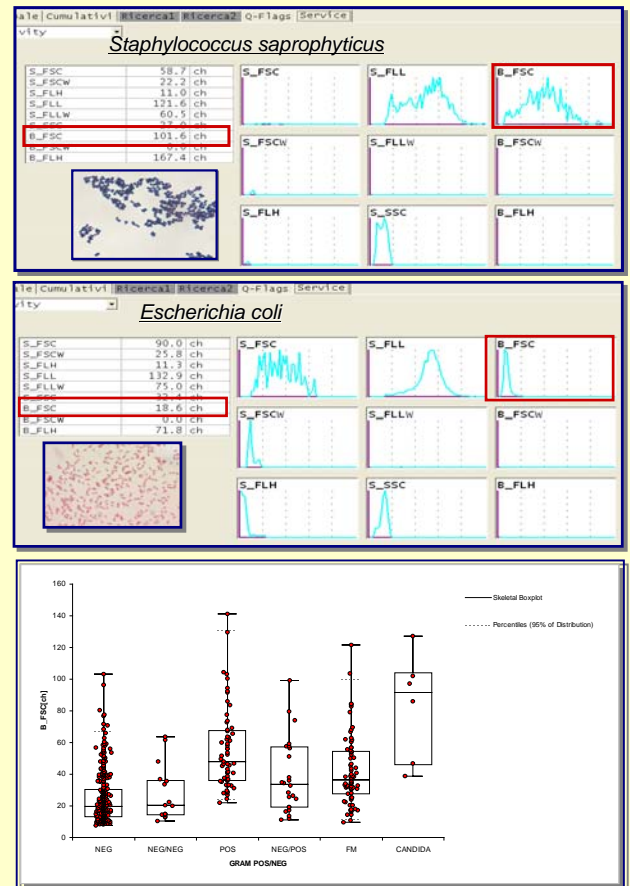


Figure 1.

Distribution of B_FSC values in 345 Gram negative and 61 Gram positive bacterial strains obtained from 515 culture positive samples. Boxes represent quartile ranges of B_FSC; a horizontal line represents the median value.

References.

1. F. Manoni et al. Cutoff values for bacteria and leucocytes for urine flow cytometer Sysmex UF-1000i in urinary tract infections. *Diagn Microbiol and Infect Dis* 65 (2009) 103–7
2. R. De Rosa et al. Evaluation of the Sysmex UF1000i flow cytometer for ruling out bacterial urinary tract infection. *Clin Chim Acta* 411 (2010) 1137–42.