Performance of Urinary Flow Cytometry in Predicting Outcome of Urine Cures

Zaman et al. reported an unsatisfactory performance of the UF-100 flow cytometer and urine strips in predicting the outcome of urine cultures in urinary tract infection (UTI) (9). However, for correct interpretation of flow cytometry data, one has to pay attention to the particular technical and preanalytical conditions and pitfalls of flow cytometry and the appropriate software to optimize UTI screening.

A critical point is the use of collection tubes adapted for pneumatic transport systems. Limited vacuum aspiration is sufficient to destroy brittle structures, increasing the amount of debris and contributing to electronic background “noise” (1).

Two different UF-100 software versions (original up to version 00-12, revised up to version 00-14) are currently being used. The algorithms for the detection of bacteria (“BACT” channel) differ between the two versions. “H-BACT,” a highly sensitive bacterial count, is displayed separately in the original version. Zaman et al. (9) used the revised version, in which the former H-BACT particles are added to the BACT channel of the instrument to generate one single result. The revised apparent BACT count is approximately 20 times higher than the original BACT count. Due to this difference, the BACT channel, lowering of the electronic threshold in this channel makes the system more sensitive to noise. Thrombocytes and debris may account for false-positive bacterial counts in cerebrospinal fluid samples (8).

We have compared both software versions using a set of 46 samples. Data for bacteria obtained by the two algorithms are not comparable in UTI screening. By using the former version, the following relationship between the white blood cell (WBC) count (y, cells/microliter) and the BACT count (x, cells/microliter) was obtained: log y = 0.914 log x − 0.989 (with r = 0.737). For the new algorithm, the correlation was weaker: log y = 0.977 log x − 2.140 (with r = 0.598). The poorer performance of the new software was due to the fact that small debris particles were considered microorganisms.

Okada et al. (using the new software) calculated a sensitivity of 83% and a specificity of 76.4% (6). Differences in the diagnostic capabilities of flow cytometry in earlier studies (sensitivity, 55%; specificity, 90%) (2) can be explained by the different software versions used. Zaman et al. reported a low sensitivity for UTI screening. However, the consideration of flagging was ignored. The UF-100 analyzer is able to detect interference with erythrocyte, crystal, and yeast cell counts, which might lead to a misclassification of these elements and an underestimation of bacteria. This is then flagged appropriately. When no attention has been paid to flagging, some false-negative results may arise. Furthermore, “positive” or “low reliable” flags for UTI are generated by UF-100 based on a comprehensive judgment of the relevant data provided by bacterial count, bacterial size (forward scatter), and WBC count. In case a large number of nonbacterial particles are detected as BACT by UF-100, the judgment of this three-parameter-rule system is low reliable.

Manufacturer-set reference values should not be confused with cutoff values. Attempts to establish reference values by an international multicenter study have failed because of preanalytical differences. Very low cutoffs for pathogenicity were suggested by Zaman et al. compared to those found in other literature (3, 7).

Zaman et al. excluded samples based only on culture results. Definition of a positive culture does not comply with particle analysis but is a combination of microbiological colony counting and clinical validation. Samples with contamination had been excluded. But the UF-100 counts dead and viable bacteria (4), regardless of contamination (mixed growth) or infection (pure colony).

The preanalytical phase is extremely important in urinalysis (4, 5). The flow cytometric evaluation of additional results for various cell types offers great help in identifying samples of poor preanalytical quality that have been received. Both epithelial cell counts and the WBC/BACT ratio may be helpful. Moreover, flow cytometry offers the advantage of measuring conductivity as well (4, 5, 7), allowing us to correct the effect of sample dilution on cell counts.

Although urine flow cytometry is not a perfect technique, the additional data provided by this novel technology allow a more balanced interpretation of the apparent bacterial count. For better prediction of UTI, algorithms using a narrower gate for the bacterial channel are preferred.

REFERENCES


Authors’ Reply

In our article (2), the reason for calling the performance of the UF-100 analyzer and urine strips in predicting the outcome of urine cultures “unsatisfactory” was not that there were high numbers of false-positive results but that for 9 out of 24 patients with false-negative results, clinically significant bacteriuria (i.e., UTI) was present. Bearing in mind the clinical consequences of not identifying UTIs, in our opinion, this miss rate was too high. Had De Langhe et al. appreciated this, much of what they have written would cease to be pertinent, for it would have led to a higher number of false-positive results. However, we are happy to acknowledge that the letter is a good exposition of the authors’ experience with urine flow cytometry. Specimens transported in a pneumatic tube system are subjected to vigorous shaking produced by sudden accelerations and decelerations. At high speeds, these have had a deleterious effect on the cellular components and some formed elements of blood and urine. We have found, however (3), that at a speed of ~4 m/s, this interference can be avoided.

De Langhe et al. contend that we ignored “the consideration of flagging.” It is not clear, even from the context, precisely to which “flagging” they refer. All the flags relevant to bacterial counts and UTI (e.g., WBC, leukocyte esterase positivity, nitrite positivity), including the presence of yeast cells, were taken into account.

As De Langhe et al. well know, for calculating diagnostic sensitivity and specificity, one needs a cutoff(s). It is in this context that different cutoffs for bacteria were used. This, we believe, was quite clear from the table.

In the clinical microbiological practice, commonly used pathogenicity cutoffs are $10^4$ and $10^5$ bacteria (CFU) per ml. For special cases, as noted in the article, $10^3$ bacteria/ml is also used (1; J. A. Washington, Letter, Arch. Pathol. Lab. Med. 122:120-122, 1998).

Although we are well aware of the preanalytical and analytical problems associated with urine specimens and of the potentials of flow cytometry, we appreciate the highlighting of these by De Langhe and colleagues.

REFERENCES