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### How did we miss it?

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## Letter to the Editor

Eline S. Andersen\*, Claus L. Brasen, Anne F. Christensen, Claus Østergaard and Ivan Brandslund

# Carryover issues with UF-5000 urine flow cytometry – how did we miss it?

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To the Editor,

We have recently evaluated the automated urine flow cytometer UF-5000 (Sysmex Corporation, Kobe, Japan) and found unexpected amounts of carryover of bacteria on the instrument. This prompted us to quantify the carryover of bacteria on the instrument as well as to critically review the commonly used metric for evaluating carryover. Our results and considerations are described in the following.

The UF-5000 is a flow cytometer, which amongst other parameters reports concentrations of bacteria and leucocytes in urine. Based on these parameters, the UF-5000 has been suggested to be able to rule out urinary tract infection and hence reduce the number of urine cultures necessary [1–3] and even guide initial therapy [1]. For this purpose, the suggested cut-off values in the literature for this instrument are 15 bacteria/ $\mu\text{L}$  (equivalent to  $15 \times 10^6/\text{L}$ ) [3], 30 bacteria/ $\mu\text{L}$  [1] and 58 bacteria/ $\mu\text{L}$  [2].

The UF-5000 has programmable automatic rinsing, and when we detected carryover, we therefore decided

to quantify the carryover of bacteria on the instrument using different automated rinse settings and different sample bacterial concentrations. This was done by analysing samples of isotonic saline (sodium chloride 9 mg/mL, sterile, Fresenius Kabi, Sèvres, France) spiked with *Escherichia coli* followed by one or more samples of sterile isotonic saline. As sterile saline has a value of zero bacteria/ $\mu\text{L}$ , the bacterial count in a sterile sample was considered to represent the amount of carryover present in that sample.

Carryover on the first sample following a contaminated sample was tested by alternately running spiked and sterile samples.

First, a spiked sample of approximately  $10^2$  bacteria/ $\mu\text{L}$  was run followed by a sterile saline sample. This process was repeated until 10 spiked samples of this concentration and 10 sterile samples had been run. After this, the maintenance operation “rinsing” was performed (an 8-min rinsing procedure).

Next, a spiked sample of  $10^3$  bacteria/ $\mu\text{L}$  was tested in the same way. As the maximum carryover observed exceeded 10 bacteria/ $\mu\text{L}$ , the test was repeated with the addition of one rinse after each spiked sample, before the analysis of the subsequent sterile sample.

The same procedure was followed for spiked samples of  $10^4$  and  $10^5$  bacteria/ $\mu\text{L}$ .

In each case, using zero rinses was tested first and number of rinses increased until the maximum carryover was less than 10 bacteria/ $\mu\text{L}$ . For each combination of sample concentration and rinse settings, 10 spiked and 10 sterile samples were run. “Rinsing” was performed before the first run of each level of spiked sample.

Table 1 shows median and maximum amounts of carryover depending on rinse settings and spiked sample bacterial count.

We also wished to know whether a carryover might affect multiple subsequent samples. To test this, we ran a single sample of just below  $10^5$  bacteria/ $\mu\text{L}$  twice followed by several sterile saline samples. We did this using the default settings of the instrument and rinse settings recommended by Sysmex, respectively. This effectively resulted

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**Table 1:** Carryover (bacteria/μL) depending on rinse settings.

Spiked sample (bacteria/μL, median)	0 Rinses	1 Rinse	2 Rinses	3 Rinses	4 Rinses	5 Rinses	6 Rinses	7 Rinses
97	2.4 (4.8)							
1000	9.6 (25)	1.2 (2.4)						
12,000	51 (61)	2.4 (8.4)						
91,000	420 (2700)	86 (1700)	49 (92)	47 (96)	15 (20)	7.2 (16)	13 (19)	3.5 (4.8)

Median (maximum observed).

in zero and two rinses after spiked samples, respectively. Figure 1 shows the results of these experiments.

As shown in Table 1, the first sterile sample following a spiked sample of just below 10<sup>5</sup> bacteria/μL yielded results well above the limit of blank reported by the manufacturer (1 bacteria/μL or less [4]) as well as above several of the cut-off values suggested in the literature for detection of bacteriuria on the UF-5000 [1–3]. This was the case whether using no rinsing (default settings of the instrument) or two rinses (settings recommended by the manufacturer). Hence, we conclude that the amount of carryover present when using default or manufacturer-recommended settings is enough to cause false-positive results and thus reduced specificity of the analysis.

We, however, also see that median carryover from samples of up to 10<sup>5</sup> bacteria/μL could be reduced to less than 5 bacteria/μL by increasing the number of rinses performed, though this will cause increased TAT and reagent consumption.

Hence, we conclude that the carryover issues can be resolved by adjusting the automatic rinse settings, and suggest that labs using the UF-5000 should program automatic rinse settings in accordance with local quality demands.

The findings regarding carryover, described in this paper, were unexpected, as the reported levels of carryover in the literature are 0.00003 (ratio. level: 99,359 bacteria/μL) [2] and 0.00% (level: 97,805 bacteria/μL) [5], respectively. The manufacturer (Sysmex) reports carryover of up to 0.05% at 1000 bacteria/μL [4], but supplies no data for samples of higher concentrations.

There may be several reasons for the discrepancies between our findings and previously reported carryover levels, including the fact that rinse settings were not specified in any of the sources. One important reason for the discrepancies may be that the way carryover was

calculated in all three cases would not capture the true extent of the problem. Specifically, all three sources calculate carryover as  $Carryover = (L1 - L3) / (H3 - L3)$ , where three high samples are run (H1, H2, H3) followed by three low or negative samples (L1, L2, L3). But, as shown in Figure 1, the carryover from the high samples may affect multiple subsequent samples, and hence the commonly used metric for calculating carryover does not capture the true extent of the problem. In future studies, we therefore recommend that the reference value used when determining carryover (L3) should be either the known value of the low concentration material or a value obtained by analysing the material in a setting with minimal risk of contamination (for instance, after thorough rinsing).

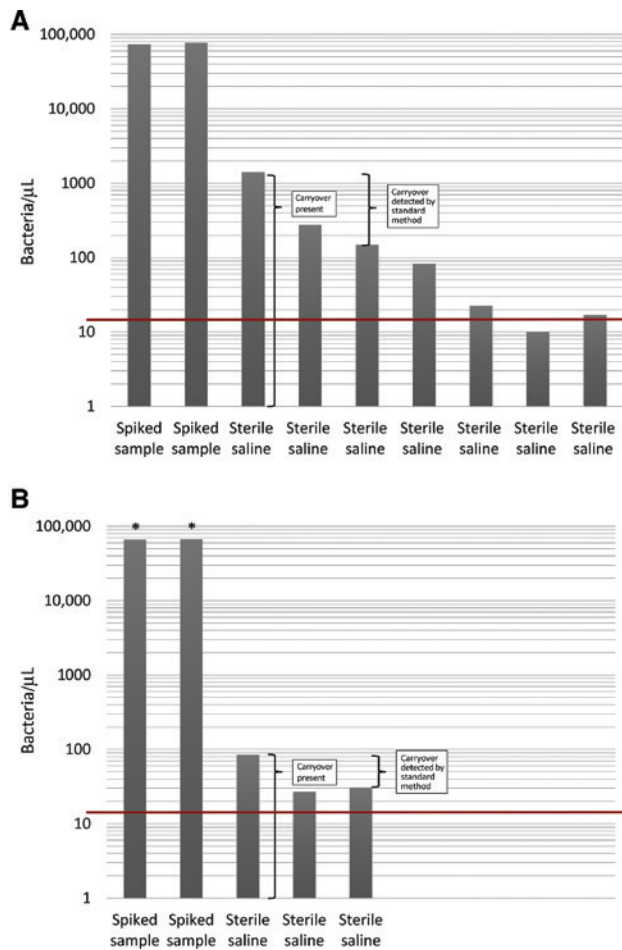
When interpreting the results of this study, several weaknesses should be considered.

All tests were performed using solutions of *E. coli* in saline, but other species of bacteria may behave differently, and future studies investigating this is warranted. Further, the iterations of the carryover experiments were performed consecutively with no rinsing between iterations apart from the rinse settings being tested. Keeping in mind that carryover might linger between iterations, the carryover on a given sample might truly be the accumulated carryover from several samples.

We report maximum observed carryover out of 10 iterations. Ten iterations are, however, not enough to reliably determine the worst-case scenario.

Based on the described findings, we suggest that any lab using the UF-5000 should set quality demands in accordance with the clinical use and choose appropriate automatic rinse settings based on tests on the relevant material.

We further suggest that when evaluating carryover, careful attention should be paid to the appropriateness of the reference value used. We recommend a known



**Figure 1:** Bacterial counts of consecutive samples of spiked and sterile saline, respectively.

The first sterile samples exceed all three recommended cut-offs from the literature for the detection of bacteriuria on the UF-5000 (15, 30 and 58 bacteria/μL, respectively [1–3]). The line represents the lowest of these cut-off values (15 bacteria/μL). \*: approximate values. (A) Default automatic rinse settings of the instrument (one rinse after samples  $>10^5$  bacteria/μL), effectively resulting in no rinses. (B) Rinse settings recommended by Sysmex (one rinse after samples of  $10^3$ – $10^4$  bacteria/μL and two rinses after samples of  $>10^4$  bacteria/μL), effectively resulting in two rinses after each spiked sample. The figure further shows the amount of carryover present and the amount detected if using the method for calculating carryover that is currently used as the standard.

reference value or a value obtained under conditions with minimal risk of contamination, which is in contrast to the widely used metric examined in this paper.

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