External Validation of rqmicro.COUNT for Bacterial Cell Count

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Abstract

An external validation of the rqmicro.COUNT instrument, a cartridge-based flow cytometer, was performed at the Universitätsklinikum Schleswig-Holstein (UKSH) in Kiel, Germany. The experiments involved the determination of the total cell count (TCC, in Ev/ml) of various aqueous solutions of both artificial and natural origin using SYBR Green DNA staining. The overall goals of the validation were to demonstrate the robustness and linearity of the instrument under field conditions and to confirm that the results obtained with the rqmicro.COUNT were comparable to those of a standard benchtop-based flow cytometer.

Introduction

The rqmicro.COUNT instrument is a very user-friendly and portable, cartridge-based flow cytometer developed by rqmicro AG in Schlieren, Switzerland. The cartridge-based system has several advantages over classical flow cytometers, which commonly use integrated fluidics with hydrodynamic focusing. It is virtually maintenance-free, does not use any sheath fluid or produce waste fluid, and does not pose any risk of carry-over from one sample to the next due to the disposable cartridge, where each sample is analysed in a dedicated channel. Additionally, it has a very small footprint (20 X 25 cm), allowing for its use where space is limited.

TCC using flow cytometry (FCM) has been proposed as an alternative to conventional plating approaches for the analysis of overall water quality (Ref.4). In general, this method involves staining microbes from an aqueous sample with a membrane-permeable fluorescent dye, such as SYBR Green DNA stain, followed by the enumeration of the stained events.

FCM offers several advantages over plating, the biggest of which is speed: While a plating-based approach can take days or even weeks to assess the presence of microbes in a given water sample due to the time it takes for visible growth to appear on the plate, TCC analysis by FCM can be performed in under 30 minutes. Additionally, many organisms present in a water sample will not be readily culturable, leading to an underestimation of the organisms present. TCC by FCM is therefore a useful, rapid method for water quality control (Ref.4).

Materials and methods

Sample preparation and SYBR Green staining

All sample preparation and analysis for the BD Accuri C6 plus were performed by Hauke Jesinghaus at UKSH. Sample preparation and staining were performed according to the standard operating procedure of the UKSH Kiel (Ref.3). Briefly, 5 µl of SYBR Green stock solution (30 mM) were added to a 500 µl sample and vortexed. The sample was then incubated at 37°C in the dark for 15 minutes, followed by analysis on the cytometer. A 50 µl sample was measured, and the result was multiplied by 20 to obtain the counts for 1 ml.

For the analysis on the rqmicro.COUNT (v0.9) instrument, minor adjustments to the above-mentioned protocol were made. For determination of the baseline and the serial dilution experiments, 20 µl of SYBR Green (100 x dilution of Molecular Probes stock solution) and 20 µl of sterile 0.5 M EDTA were added to a 2 ml sample, mixed well and incubated at 37°C in the dark for 15 minutes, before being directly loaded into the rqmicro.COUNT cartridge. For the robustness analysis and the small field trial of 20 independent water samples, Tween 80 was added to a final concentration of 0.05% (v/v) at the same time as the other reagents. A 180 µl sample was measured, and the result was multiplied by 5.55 to obtain the counts for 1 ml.

Results

Figure 1 illustrates the appearance of the plots and the gating strategy used for the two instruments. The drinking water sample (LU1052262) came from routine analyses carried out in the UKSH laboratory. The red gate defines the area of the counted bacteria.

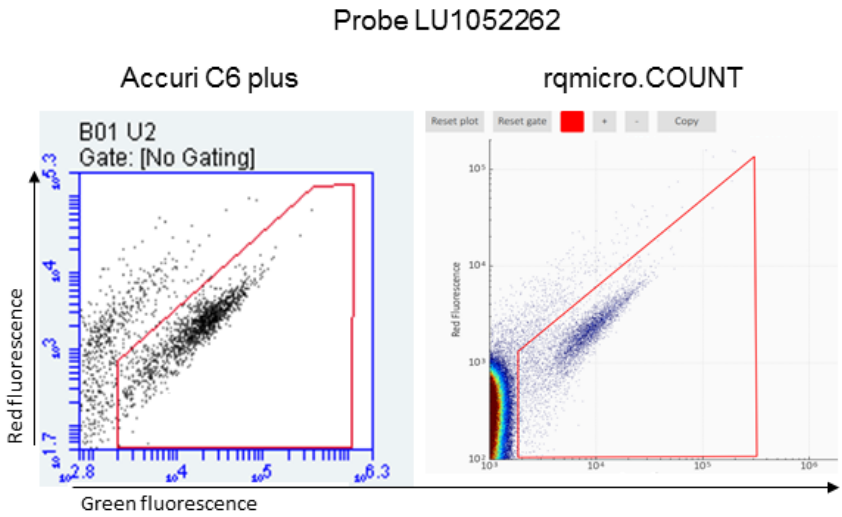

Figure 1: Illustration of the appearance of the plots and gating strategies used on the two instruments.

Determination of the limits of detection (LoD) and quantification (LoQ)

To determine the limits of detection and quantification, filtered (0.22 µm pore size) ultrapure water was stained with SYBR Green, and 8 technical replicates were analysed on both instruments. The mean and standard deviations of the measurements were calculated. The LoD was defined as 3 times the standard deviation and the LoQ as 9 times the standard deviation, according to DIN 32645 (Table 1).

Table 1: Mean and standard deviations, LoD and LoQ based on the measurements of 8 technical replicates of ultrapure water:

Limit of Detection	Mean Ev/ml	Standard deviation	LoD (Ev/ml)	LoQ (Ev/ml)
Accuri C6 plus	5.0	9.3	28	83
rqmicro.COUNT	14	12	37	111

Robustness

To investigate the robustness of the measurements obtained with the rqmicro.COUNT instrument compared to those of the BD Accuri C6 plus, two independent water samples were analysed. One sample was drawn from a cold-water tap in the UKSH building (KW209) and the other one had been sent to UKSH for analysis (LU1052262). Both samples were stained with SYBR Green according to the two protocols, and 8 technical replicates of each sample were analysed on the two instruments.

The mean and standard deviations for both samples and instruments were calculated and plotted as box plots (Figure 2). For both water samples tested, the TCC numbers obtained with the BD Accuri C6 plus showed smaller standard deviations compared to the numbers obtained with the rqmicro.COUNT.

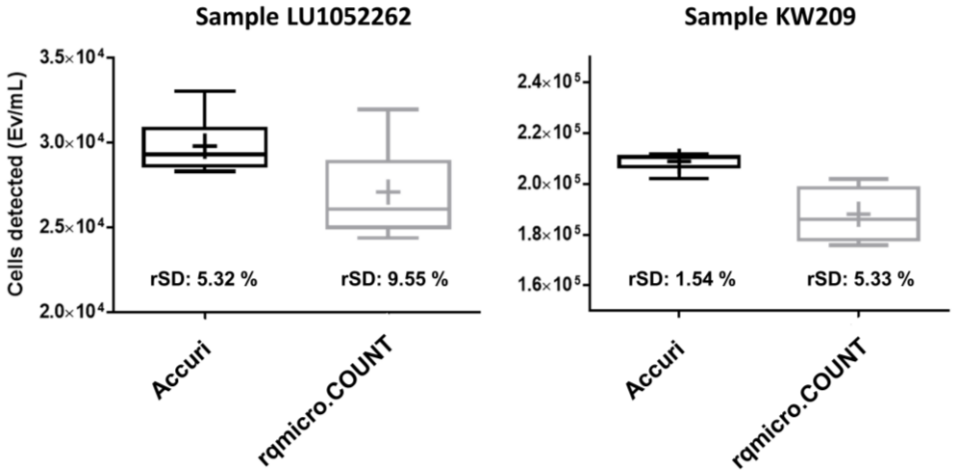

Figure 2: Robustness analysis of each instrument. Mean values and the relative standard deviation are shown.

To directly compare the results between the two instruments, the percentage deviation of the mean values μ_{TCC} for both samples was calculated according to the following formula: (μ(Accuri) - μ(rqmicro.COUNT))/((μ(Accuri) + μ(rqmicro.COUNT))/2*100; see Table 2.

Table 2: Deviation between the two instruments:

Sample	Percentage deviation
LU1052262	9.5%
KW209	10%

The deviation between the two instruments for the 2 water samples was calculated as 9.5% and 10%, respectively. The results were slightly higher when the samples were analysed on the BD Accuri C6 Plus.

Linearity

To investigate the linearity and linear range of the rqmicro.COUNT instrument compared to the BD Accuri C6 plus, two different dilution series were prepared, then stained with SYBR Green according to the two protocols and analysed on both instruments. Each dilution point was analysed in duplicate on both instruments.

The first dilution series was performed with a water sample drawn from a tap at UKSH (KW109). The sample was diluted in 1:10 steps with 0.8% NaCl down to 10^{-3} (Figure 3).

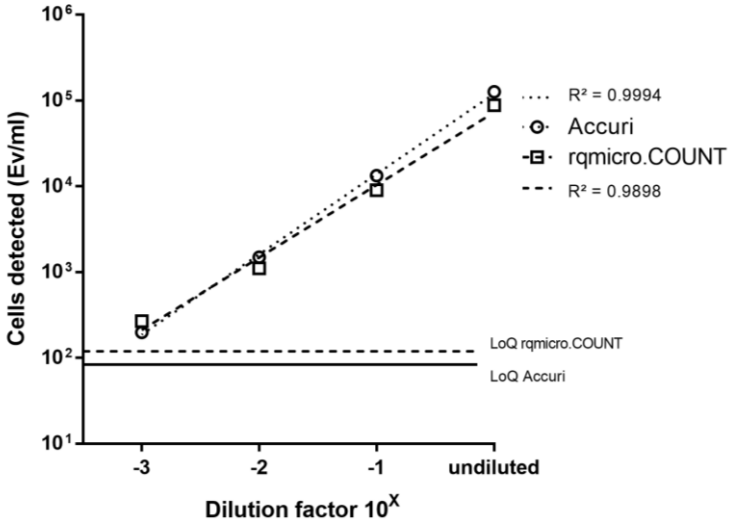

Figure 3: Analysis of instrument linearity by measuring a dilution series of tap water (KW109) with both the BD Accuri C6 plus and the rqmicro.COUNT.

The second dilution series was performed with an overnight bacteria culture. The culture was diluted in 1:10 steps with 0.8% NaCl down to 10^{-8}, and dilutions 10^{-1} to 10^{-8} were analysed on both instruments.

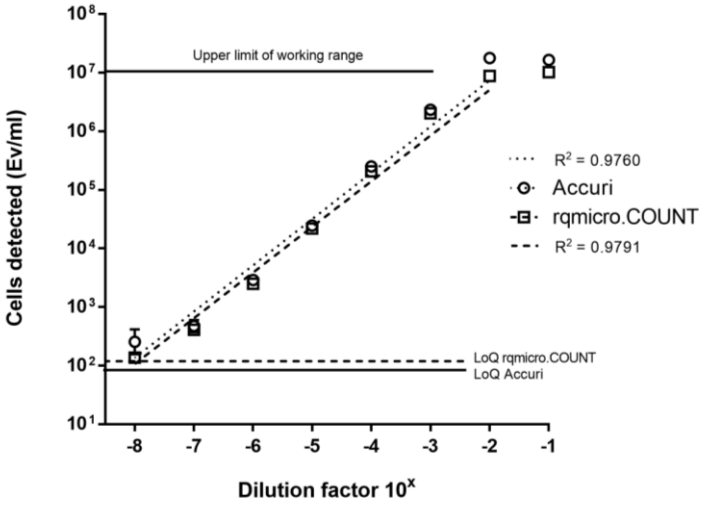

Figure 4: Analysis of instrument linearity by measuring the dilution series of an overnight bacterial culture with both the BD Accuri C6 plus and the rqmicro.COUNT.

The analysis of both dilution series clearly demonstrated linearity for the rqmicro.COUNT instrument, starting above the previously calculated LoD and up to approximately $1x10^7$. This range was comparable to the BD Accuri C6 plus.

In a further experiment, the concentration of an overnight bacterial culture was first determined by TCC measurement on the BD Accuri C6 Plus, and a specified number of these bacteria was then spiked into tap water sample KW209 at four different spiking levels and analysed in duplicates on both instruments. In order to investigate whether the spiked bacterial cells could be detected in addition to the naturally occurring bacteria, the previously determined number of bacteria, which was approximately 188,000 cells/ml for the rqmicro.COUNT and 209,000 cells/ml for the Accuri, was subtracted (equal to the mean values of KW209 shown in Figure 2). Using this approach, the additional bacterial cells were successfully detected on the BD Accuri C6 plus at all four spiking levels, and on the rqmicro.COUNT at the 20,000, 50,000 and 100,000 spiking levels (Figure 5).

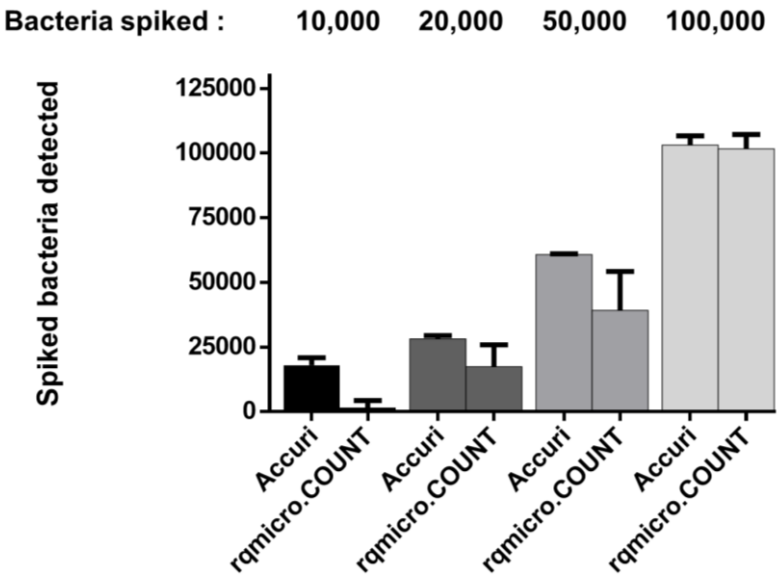

Figure 5: Detection of additional bacteria spiked into tap water sample KW209. Additional bacterial cells were spiked into KW209 at the indicated levels and the samples were then analysed on both instruments.

It is possible that the lowest spiking level was not effectively detectable on the rqmicro.COUNT because it falls within the standard deviation of the instrument. However, it was possible to detect the additional spiked cells with the rqmicro.COUNT at the higher levels.

Field study with 20 water samples

To compare the performance of the rqmicro.COUNT with the BD Accuri C6 plus across multiple samples, a small field trial with 20 different water samples was performed. All samples were stained with SYBR Green according to the two protocols and analysed on both instruments. For this experiment, each sample was only measured once (Figure 6). 4 out of these 20 samples were below the detection limit of the instrument (Ref.3) and were thus excluded from further analysis. A comparison between the values obtained on the two instruments demonstrated a good correlation between the numbers (R^2=0.9728). The mean percentage deviation between both instruments for these measurements was 22%, which

was calculated in the following way: $\frac{1}{n}\sum_{i=1}^{n}|((\mathrm{xi}-\mathrm{yi})/((\mathrm{xi}+\mathrm{yi})/2))*100|$ (x_i=result Accuri, y_i=result rqmicro.COUNT).

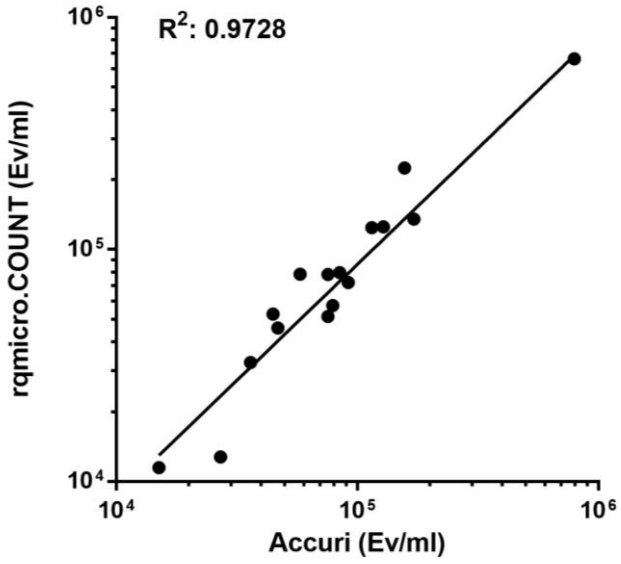

Figure 6: Comparison between total cell count measurements obtained on the rqmicro.COUNT and the BD Accuri C6 plus for 16 different water samples with quantifiable results.

Conclusions

The overall goal of this validation was to assess the performance of the rqmicro.COUNT flow cytometer with regards to sensitivity, robustness and linearity compared to a standard benchtop instrument – in this case, the BD Accuri C6 plus.

In terms of sensitivity, the rqmicro.COUNT performed very well and in a comparable manner to the BD Accuri C6 plus, with a low limit of detection of around 40 cells/ml using filtered distilled water. In terms of robustness, the rqmicro.COUNT showed higher variability in the standard deviation for two water samples. However, the standard deviation was below 10% and therefore suitable for reliable TCC analysis, as well as falling within the deviation of the BD Accuri C6 plus in routine analysis of tap water. In addition, the values obtained with the rqmicro.COUNT during the robustness study were approximately 10% lower compared to those obtained with the BD Accuri C6 plus. In terms of linearity, the rqmicro.COUNT showed very good results, with a linear range from near the limit of detection up to $1x10^7$ cells/ml. These results are comparable with the performance of the Accuri C6 plus. As such, both instruments offer a detection range that is more than sufficient for drinking water analysis. The next step will be additional testing of the rqmicro.COUNT with further samples, e.g. tap water from the drinking water systems of different buildings carried out by a third person in the field.

In conclusion, the sensitivity, robustness and linearity data demonstrate that the rqmicro.COUNT flow cytometer is a reliable instrument for the determination of TCC numbers in drinking water. Overall, the results obtained with the rqmicro.COUNT flow cytometer correlate well with the results obtained with a research-grade flow cytometer.

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