Explainer #1: CytoQuant® Proof-of-Principle Study. Comparison with Plate Counts

Summary

A microbiological proof-of-principle study was conducted to assess the performance of the CytoQuant® mobile impedance flow cytometer against two other methods for quantifying microbial contamination: a total viable count on Tryptone Soya Agar (TSA) and an enhanced total viable count on Tryptone Soya Agar with 2,3,5-triphenyltetrazolium chloride (TSA+TTC).

Overall, comparison of data shows a strong positive correlation between CytoQuant® and the other methods of quantifying bacterial contamination. Only 1 sample within the detection limits of the device was outside of 1 log variance. The correlation between TTC enhanced TSA count and CytoQuant® was 0.978 and that between CytoQuant® and the regular TSA count was 0.984.

Materials and Methods

Microorganism and inoculum preparation

Cultures in table 1 were taken from frozen cryobead storage at -80°C, used to inoculate a pre-poured TSA plate and incubated at 37°C overnight. Colonies were used to inoculate another plate, creating a second subculture. Cultures were examined to confirm purity and colony morphology, then colonies were mixed in 5% Phosphate Buffered Saline (PBS) to produce an inoculum suspension for each organism.

Table 1. Microorganisms included in the study.

Microorganism	Reference
Salmonella enterica ser. Typhimurium	NCTC 74
Staphylococcus aureus	ATCC 6538
Pseudomonas aeruginosa	ATCC 15442
Listeria monocytogenes	NCTC 11994
Bacillus cereus	NCIMB 11925
Escherichia coli	NCTC 10418
Pediococcus acidilactici	NCIMB 7881

An inoculum was produced for each organism and combined to obtain cocktail inoculum of low and high levels: approximately 10^4 and 10^6 colony forming units (CFU) per mL, respectively.

The following uninoculated food samples containing naturally occurring microflora as listed below, were also used in the study:

- Fish juice (from pollock)
- Meat juice (from diced chicken breast)
- Mozzarella brine (juice)
- Lettuce rinse

The excess liquid from inside packeted cheese or meat products was diluted using PBS. Lettuce leaves were stomached (10 grams per 100 mL PBS) to suspend microflora as 'lettuce rinse' and then diluted further as detailed below. All samples were diluted as described below, to achieve approximately 10^4 and 10^6 CFU/mL and then mixed thoroughly.

- Fish juice diluted to 1:100 and 1:10,000 for high and low inoculums, respectively.
- Meat juice diluted to 1:10,000 and 1:100,000 for high and low inoculums, respectively.
- Mozzarella juice diluted to 1:1000 and 1:10,000 for high and low inoculums, respectively.
- Lettuce rinse diluted to 1:100 and 1:1000 for high and low inoculums, respectively.

Plate Counts
A total viable count is a method used to estimate the number of live microorganisms in a sample. After preparation, samples were serially diluted with maximum recovery diluent (MRD) and 1 mL aliquots of each dilution were transferred into duplicate sterile Petri dishes. Approximately 15 mL molten TSA was added, plates were mixed, inverted, and then incubated at 30°C ± 1°C for 48 hours.

TTC staining is used as a cell counting aid, TTC is reduced by active enzymes in metabolically active cells to produce a red pigment and limits the spreading of colonies. After preparation, samples were serially diluted with MRD, and 1 mL aliquots of each dilution were transferred into duplicate sterile Petri dishes. Approximately 15 mL molten TSA (mixed with 1 vial of 1% TTC per 500 mL agar) was added, plates were mixed, inverted, and then incubated at 30°C ± 1°C for 48 hours. Colonies with red coloration were counted.

CytoQuant®
The CytoQuant® mobile flow cytometer measures fluctuations in impedance caused by viable (membrane intact) bacterial cells by passing the sample through a microfluidic flow cell with integrated electrodes. Readings take 30 seconds or 2 minutes, depending on the desired sensitivity or precision. Two distinct results are produced, the first result is a count of viable microorganisms (used for this study), while the second is a count of other similarly sized particles, both counts must fall within range for the device to provide a quantifiable reading.

Amounts of 3 mL sample were added to each measuring vial. Samples were processed within 45 minutes. Lower inoculum levels were processed using the 2-minute read. Higher inoculum levels were processed using the 30-second read. The device has a lower limit of detection of 5,000 intact cells per mL and an upper limit of 10,000,000 intact cells per mL.

To assess the variability between devices, three devices were used in the study. Prior to commencement of the study each device was 'qualified' (calibration verification) against a reference sample of known quantity.

Results

Of the five sample types, only three produced numeric results – and thus could be plotted (figure 1) – for all three replicates: cocktail inoculum, fish juice and lettuce rinse. The mozzarella brine and meat juice samples produced CFU counts that were significantly lower than expected (2 log or lower), with CytoQuant® displaying results below the LOD for most samples.

The output of the three methods was strongly correlated (table 2), with data showing close agreement. As expected, given that fresh cultures were used, the results pertaining to the cocktail inoculum samples were closest (variances <0.3 log). The highest variance was observed for the mozzarella brine samples (>2 log) and lettuce rinse samples (>1 log).

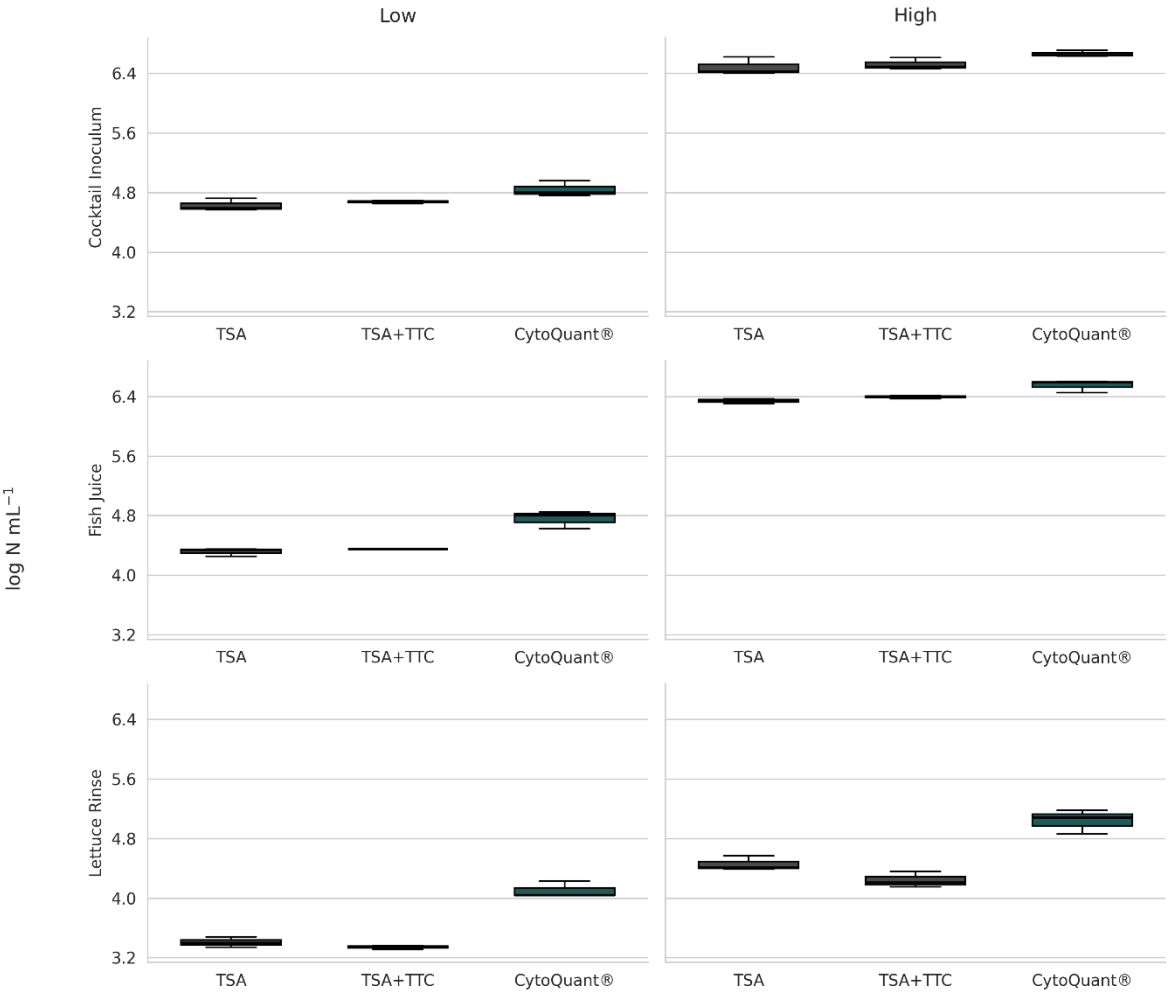

Figure 2. Boxplots of the total viable counts provided by the three methods used in the study: plating on Tryptone Soya Agar (TSA), with or without 2,3,5-triphenyltetrazolium chloride (TTC), and CytoQuant® impedance flow cytometry. Only full sets (i.e., with numeric results for each of the three replicates) of data were plotted.

For all measured samples, CytoQuant® results were higher than those of the plate counts. This positive bias, smallest for the cocktail inoculum samples and highest for the mozzarella brine samples, was likely brought about by the ability of the instrument to detect and count cells irrespective of their ability to grow and develop into a colony. Cheese samples are known to include high numbers of viable but not culturable microorganisms, due to pH conditions and the existence of an inhibiting/bioprotective background microbiota (starter cultures are used in production for their ability to lower pH and secrete antimicrobials that inhibit spoilage microorganisms). The somewhat lower bias observed for the fish juice samples is likely explained by the higher abundance of spoilage microorganism (species that grow in the matrix during storage, thus culturable).

Table 2. Correlation coefficients.

	TSA	TSA+TTC
CytoQuant®	0.984	0.978
TSA	-	0.995

Table 3. Results for the naturally contaminated mozzarella brine samples.

Inoculum level		High			Low		
	Method	TSA	TSA+TTC	CytoQuant®	TSA	TSA+TTC	CytoQuant®
log N mL^{-1}	R1	2.44	0.00	4.51	1.12	0.00	<3.70
	R2	2.38	0.18	4.18	1.22	0.00	<3.70
	R3	2.34	<0.00	<4.18	1.24	<0.00	<3.70
	AVG	2.39	0.06	4.35	1.19	0.00	-
	STDEV	0.0411	-	-	0.0525	-	-

R1, R2 and R3 – replicates; AVG – average; STDEV – standard deviation.

Table 4. Results for the naturally contaminated meat juice samples.

Inoculum level		High			Low		
	Method	TSA	TSA+TTC	CytoQuant®	TSA	TSA+TTC	CytoQuant®
log N mL^{-1}	R1	2.54	2.00	<4.18	1.88	1.54	<3.70
	R2	2.54	2.54	<4.18	1.40	1.40	<3.70
	R3	2.48	2.60	<4.18	1.40	1.18	<3.70
	AVG	2.52	2.38	-	1.56	1.37	-
	STDEV	0.0283	0.2698	-	0.2263	0.1482	-

R1, R2 and R3 – replicates; AVG – average; STDEV – standard deviation.

Conclusions

The CytoQuant® devices offered a quick alternative to enumeration within their defined limits of determination, with data showing a strong (>0.978) positive correlation between the counts of the tested methods of quantifying bacterial contamination. Only 1 sample within the detection limits of the device was outside of 1 log variance, with CytoQuant® counts being higher than the plate counts. In the case of naturally contaminated samples higher in unculturable or slowly growing microorganisms, this positive bias is expected to increase.

Important:

Please read the product documentation before performing any measurements. User manuals, instructions, certificates of analysis and other documents can be downloaded by visiting
https://www.romerlabs.com/en/customer-resources/

For the full scientific report, please contact your Romer Labs representative.