Red One™
Instantaneous & quantitative microbial detection in food matrices
Feasibility study on a dairy matrix: Total Viable Counts in raw milk cheese

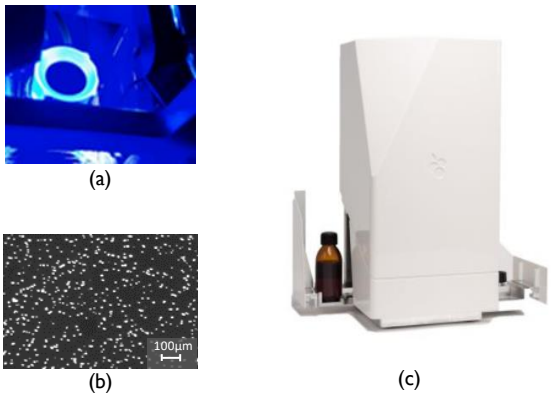
Detecting and counting accurately and rapidly microorganisms in water, food, pharmaceutical or biomedical products is a current challenge to ensure reliable and rapid quality controls. Traditional standard plate count methods usually require a minimum of 2 to 3 days before delivering a result for Total Viable Counts (TVC).
In the food industry, raw milk and fermented food products present a high microbial load and diversity. Controlling rapidly their microbial load, as a quality indicator, is essential to define the food quality, besides the food safety.
This is one of the applications for which Red One™ demonstrated promising results presented in this application note in comparison with another well-established alternative method for aerobic mesophilic count. Total viable counts obtained on raw milk cheese samples with Red One™ show a strong correlation (R^2>0.94) with Petrifilm™ Aerobic Count (AC) Plate method (ISO-16140 Part 2 and AOAC validated).
Red One™ direct counting accuracy is confirmed in the range of 10-300 CFU/mL (up to 10^6 CFU/mL).

Red One™ patented platform

Red One™ is a fully automated rapid microbiology system based on solid-phase cytometry technology. It detects single cell microorganisms and delivers instantaneous and quantitative microbiological results.
Results are obtained rapidly (less than 10 minutes). The system is easy to use in a two-step process:

- the sample is dropped onto a cap
- the sample is filtered through a membrane.

A fluorophore (agent) is then automatically injected to stain live microorganisms. Redberry dye reveals fluorescence relating to cellular metabolism. Staining kinetics (the evolution of fluorescence over time) is analyzed to differentiate microorganisms from inert particles. Every step, from sample preparation to analysis is automated in a streamlined workflow: filtration, staining, image acquisition and cell counting.



(a) (b) (c)

Figure 1 – (a) Red Caps under 480 nm excitation, (b) High Resolution picture of *Saccharomyces cerevisiae*, (c) Red One™ System

The staining agent RedChrome V1, an esterase sensitive fluorophore, is minimally toxic to living cells, allowing an accurate and reliable marking.

Testing workflow and protocol

Tested matrix is a well-known French cheese called "Camembert" (made with raw milk). Sample preparation consists of the following steps:

- 25g of cheese mass are re-suspended in 225 mL of peptone water in a blender bag with filter (20 µm porosity) and homogenized with a lab blender
- The solution is then diluted with tryptone salt.

Samples are cultivated and then counted with Petrifilms according to the standard workflow [1]: inoculation with 1 mL of the sample, then cell counting at 72 hours after incubation at 30°C. Analysis is performed simultaneously within 10 minutes (*vs.* 72h) with Red One™: 1 mL of the targeted dilution is directly dropped on a cap and then analyzed.
Both workflows are described in Figure 2. More information on respective methods can be found on:
3M Petrifilm Aerobic Count Plates - Plating Technique: https://vimeo.com/43670756
Red One - Rapid Microbiology System: https://youtu.be/9MvN_QatZ0k

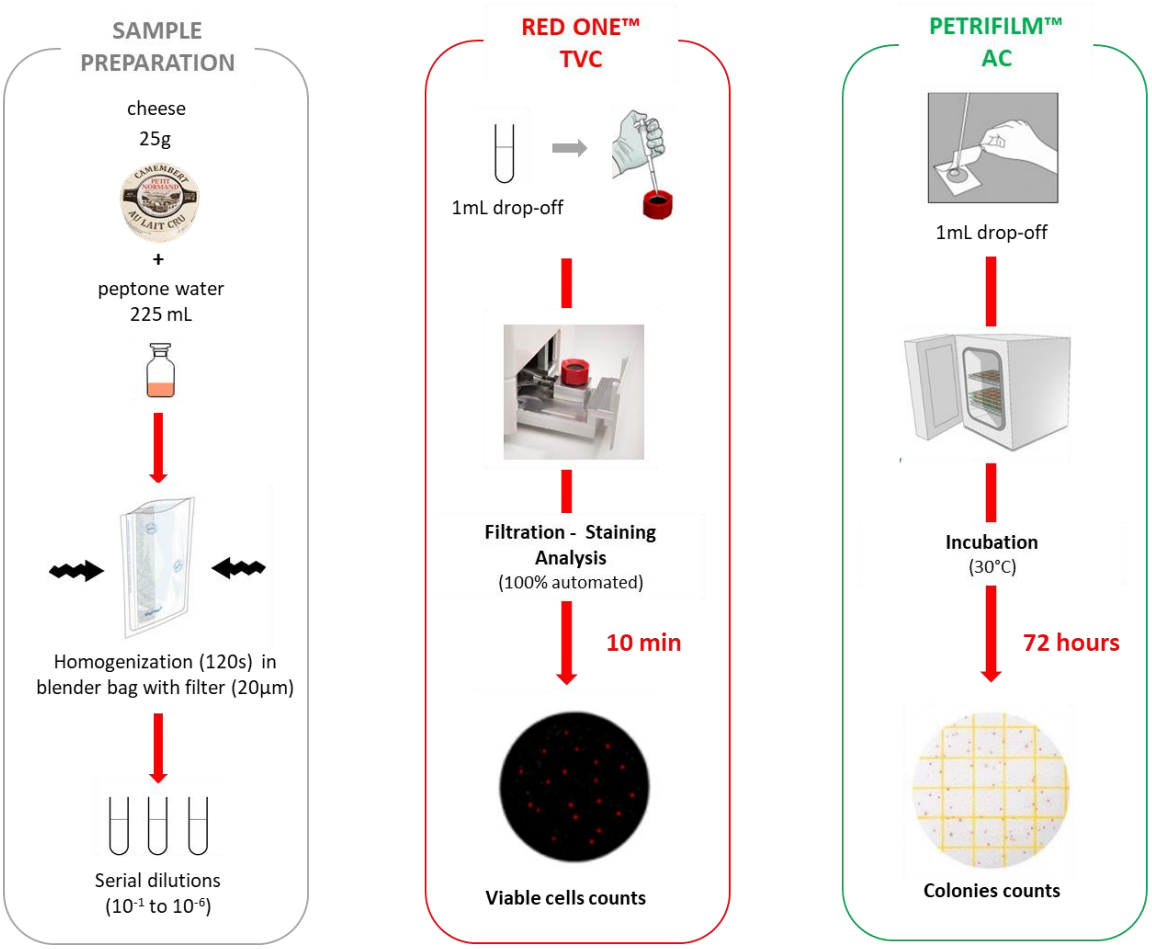


Figure 2: Testing Workflows

Counting limits of Petrifilm™ and Red One™

With Petrifilm™ AC method, counting range has to be less or equal to 300 colonies (Figure 4). Therefore, only 10^{-5} and 10^{-6} dilutions could be counted.

With Red One™, the upper limit of detection is around 5 10^5 cells/cap. The dilutions 10^0 and 10^{-1} cannot be counted with 1 mL sample since they are too concentrated. Nevertheless, diluting the sample directly in the cap is an option: in that case 990µL of tryptone salt is dropped on a Red Cap, and 10µL of the initial solution or 10^{-1}dilution is added (Figure 3).

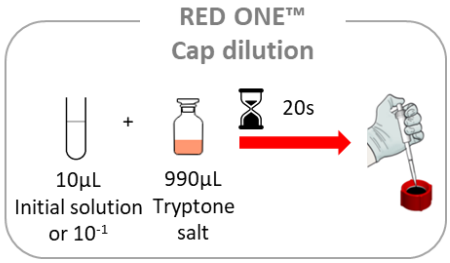


Figure 3: Dilution in caps

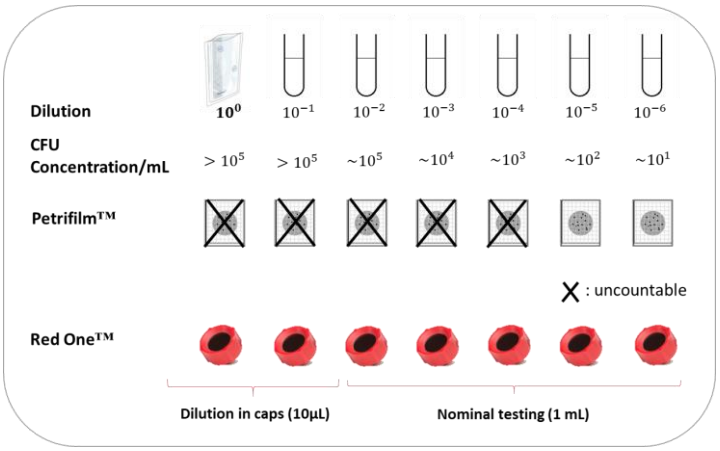


Figure 4: Dilution and counting ranges

To count Petrifilms for dilutions between 10^0 and 10^{-4}, extrapolation is done with the following formula (ISO 7218, §10.3.2.2):

$$N = \frac{\sum C}{V * 1.1 * d}$$

with:
N: number of micro-organisms
$\sum C$: sum of colonies counted on two Petrifilms of successive dilutions
V: volume tested
d: dilution factor for first dilution

It should be noted that any error of dilution or unexpected microbial load (i.e. exceeding 300 colonies) can only be detected after 72h with Petrifilm™ AC. The use of Red One™ for these cases allows to relaunch tests in the right conditions of dilution.

Enumeration results - Red One™ vs. Petrifilm™ AC

Both counting linearity and accuracy of Red One™ is analyzed by comparison with the Petrifilm™ method results. Four cheeses test units have been analyzed from 2019-11-28 to 2020-03-03 according to protocols given in Fig. 2.
31 counts with serial dilutions between 10^{-1} and 10^{-4} (not countable on Petrifilms) and 12 counts with dilutions between 10^{-5} and 10^{-6} were performed with both Red One™ and Petrifilms. As demonstrated in Fig. 5, Red One™ produced results with a strong correlation ($R^2 = 0.98$) with the Petrifilm™ method when considering all dilutions tested.

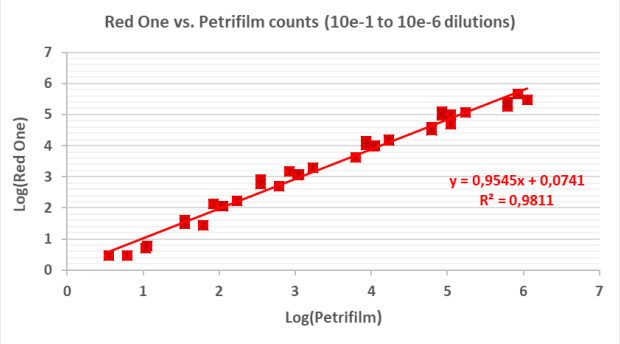


Figure 5: Correlation between Red One™ and Petrifilm™ AC counts ($\log_{10}$ CFU/mL) for dilutions from 10^{-1} to 10^{-6}

Countable dilutions (i.e. comprised between 10 and 300 CFU/mL) for both methods were also compared (Figure 6). Although these concentrations are closer to the lowest detection limit of Red One™ (10 cells/mL), the correlation between methods remains quite good ($R^2 = 0.94$).

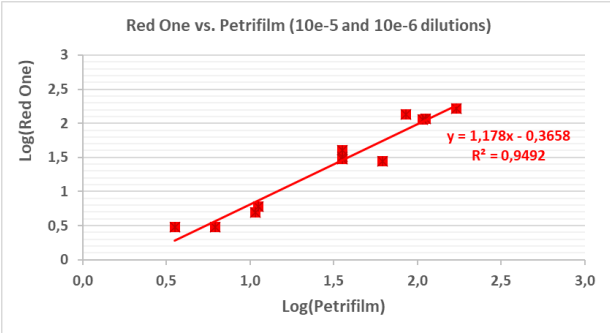


Figure 6: Correlation between Red One™ and Petrifilm™ AC counts $\log_{10}$ CFU/mL) for dilutions from 10^{-5} and 10^{-6}

A 0.3 $\log_{10}$ CFU/mL difference **of the microbial counts** between methods is a commonly acceptability limit in food microbiology. For all ranges of concentrations (low, medium & high - see appendix), differences are below 0.3 $\log_{10}$ CFU/mL on average. The difference is higher at high concentrations (0.28 $\log_{10}$) against 0.12 $\log_{10}$ for medium and 0.15 $\log_{10}$ for low concentrations since Red One™ high detection limit 10^6 CFU/mL is almost reached, which can induce some undercounting.

It is thus shown that Red One™ can accurately measure levels between 10 to 10^3 cells/mL which corresponds to a higher sensitivity than demonstrated by other rapid techniques used for dairy products. In previous studies [2, 3], ATP-metry or Flow cytometry (FCM) were also used to obtain results within minutes but it is generally admitted that detection limit for FCM is typically 10^3 to 10^4 cells/mL. Likewise, ATP-metry cannot detect lower concentrations than 10^5 cells/mL and provides an indirect result that depends of each microbial flora, therefore lacks some repeatability.

Conclusions

Red One™ counts of viable cells in a raw milk cheese sample are strongly correlated with the Petrifilm AC method (ISO-16140 Pt2 validated) for a wide range of Total Viable Counts (10 to 10^6 CFU/mL). Performances in the range of 10 to 10^3 CFU/mL confirmed the higher sensitivity of Red One™ in comparison with other rapid methods such as Flow cytometry or ATP-metry (> 10^6 CFU/mL). It makes the method particularly suitable for applications requiring low detection limits (sterilized milk, powdered products, in process control…) along with its ease-of-use and short turnaround time (results obtained in 10 minutes), allowing fast and accurate decision making.

References

[1] 3M Petrifilm Total Viable Count utilization guide. Available at http://multimedia.3m.com/mws/media/375349O/pac-interp-guide-fre.pdf
[2] Thusitha, S, et al. « A Flow Cytometry Method for Rapid Detection and Enumeration of Total Bacteria in Milk ». Applied and Environmental Microbiology, 1228-1232 (2000).
[3] M.C. te Giffel, J. Meeuwisse, P. de Jong. « Control of milk processing based on rapid detection of micro-organisms ». Food Control, Volume 12, Issue 5, July 2001, Pages 305-309.

Partnership: Aerial, Technological Resource Center and Technical Institute for Food Industry (France)
Find out more at www.redberry.net and www.aerial-crt.com

Appendix - Accuracy of Red One™ method

Table 1: Red One vs. Petrifilm counts (log_{10} CFU/mL)
Low concentrations (btw. 10e1 and 10e2 CFU/mL i.e. dilutions btw. 10e-6 and 10e-5)

Dilution factor	Red One™ count (log10)	Petrifilm count (log10)	ΔRed One™ count - Petrifilm count (log10)
1,00E-05	1,45	1,79	-0,34
1,00E-06	0,48	0,79	-0,31
1,00E-06	0,78	1,05	-0,27
1,00E-05	2,07	2,05	0,02
1,00E-05	2,22	2,24	-0,02
1,00E-05	2,13	1,93	0,20
1,00E-05	1,60	1,55	0,05
1,00E-05	1,51	1,55	-0,04
1,00E-05	1,48	1,55	-0,07
1,00E-06	0,48	0,55	-0,07
1,00E-05	2,06	2,03	0,03
1,00E-06	0,70	1,03	-0,33
Mean			**0,15**
Standard deviation			0,13

Table 2: Red One vs. Petrifilm counts (log_{10} CFU/mL)
Medium concentrations (btw. 10e3 and 10e4 CFU/mL i.e. dilutions btw. 10e-4 and 10e-3)

Dilution factor	Red One™ count (log10)	Petrifilm count (log10)	ΔRed One™ count - Petrifilm count (log10)
1,00E-03	4,15	3,93	0,22
1,00E-04	3,16	2,93	0,24
1,00E-03	4,15	3,93	0,22
1,00E-03	3,63	3,79	-0,16
1,00E-04	2,70	2,79	-0,09
1,00E-04	3,07	3,05	0,02
1,00E-03	4,01	4,05	-0,04
1,00E-04	3,29	3,24	0,05
1,00E-04	3,29	3,24	0,06
1,00E-03	4,18	4,24	-0,06
1,00E-03	4,19	4,24	-0,04
1,00E-03	4,01	3,93	0,08
1,00E-04	2,90	2,55	0,35
1,00E-04	2,77	2,55	0,22
1,00E-03	3,98	4,03	-0,05
1,00E-04	3,04	3,03	0,01
Mean			**0,12**
Standard deviation			0,10

Table 3: Red One vs. Petrifilm counts (log_{10} CFU/mL)
High concentrations (btw. 10e4 and 10e6 CFU/mL i.e. dilutions btw. 10e-2 and 10e-1)

Dilution factor	Red One™ count (log10)	Petrifilm count (log10)	ΔRed One™ count - Petrifilm count (log10)
1,00E-02	5,08	4,93	0,15
1,00E-02	5,03	4,93	0,10
1,00E-01	5,27	5,79	-0,52
1,00E-01	5,34	5,79	-0,45
1,00E-02	4,50	4,79	-0,30
1,00E-02	4,49	4,79	-0,30
1,00E-02	4,50	4,79	-0,29
1,00E-01	5,39	5,79	-0,40
1,00E-02	4,60	4,79	-0,19
1,00E-02	4,69	5,05	-0,36
1,00E-02	4,99	5,05	-0,06
1,00E-01	5,46	6,05	-0,59
1,00E-02	5,06	5,24	-0,18
1,00E-02	4,97	4,93	0,04
1,00E-01	5,66	5,93	-0,27
Mean			**0,28**
Standard deviation			0,16