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National TBC-conversion in Italy

On the road to more uniform bacteria settlement!

By

Dr Giuseppe Bolzoni and Berte Asmussen

To improve the reproducibility of flow cytometry technique for total bacteria count in milk, a conversion from instrumental results (impulses or Individual Bacteria Counts per µl) to reference results (Colony Forming Units per ml) is needed. Italy has conducted a project to develop a common national conversion line for her BactoScan FC instruments.

The trial report, which contains a wealth of interesting information for conversion aficionados, was published earlier this year in Italian Journal of Food Science (IJFS,27,1, 2015).

Dr Giuseppe Bolzoni, Izsler and Berte Asmussen, Raw Milk Connect have extracted highlights from this impressive study with 29 participating laboratories!

Figure 1 Trial report of experiments based on ISO standards no. 21187 and 4833-1

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Background and project aims

The primary project aim was to tailor a conversion equation for the entire Italy and to validate a preliminary equation produced some years earlier. The preliminary equation (2009-2010) was in particular directed at the EU-regulatory level of 100.000 CFU/ml and therefore the final equation (2011-2012) had to be boosted with high count samples.

Design of experiments

The experiment involved 29 laboratories, which recorded the Total Bacteria Count of ***unpreserved*** milk samples using both the manual reference method (ISO 4833-1) as well as the instrumental method.

Considering that ISO 19036 estimates that repeatability error of the Plate Count method originate more from the analyst than from deviations of the initial bacteria concentration, particular care was taken to circumvent this aspect. One dilution series was made from each sample and 2 plates produced from each of 3 dilution steps. Samples were analyzed ***first on the flowcytometer*** and afterwards by the reference method, both methods were performed in duplicate.

Figure 2 the 20 regions of Italy

Selection of results and validation of lab performance

With second phase of the project 1827 raw cow milk samples was analyzed: 499 or 27 % were rejected – 17% due to the usual criteria such as poor repeatability, out-of-range and unreliability, that is no correspondence between instrumental and reference results or relationship between plates and dilution in reference method (G^2 Factor test) .

Further 10% of the results were left out due to performance of individual laboratories: very large or too low Sy,x´s – see figure 3, high frequency of sub-dispersed results from the reference method or high frequency of eliminated results from a G^2 factor test.

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Lab Code	Samples (n)	Intercept	Slope	Sy:x
40	50	2.1184	1.0309	0.0139
27	98	2.9025	0.7797	0.0930
14	42	2.4432	0.9911	0.1455
38	36	2.2563	1.0279	0.1577
31	93	2.3363	1.0408	0.2517
35	52	2.1976	1.0711	0.2556
41	16	2.1718	1.0859	0.2594
1	40	2.1280	0.9966	0.2676
39	88	2.6219	0.8914	0.2766
15	26	2.5538	1.0119	0.3086
11	26	2.5408	0.9711	0.3118
23	98	2.6394	0.9257	0.3223
24	50	2.4829	0.9593	0.3291
6	68	2.2774	1.0927	0.3365
28	54	3.5260	0.6413	0.3546
37	79	3.6620	0.5508	0.3707
22	76	2.7561	0.8592	0.3756
26	55	2.1806	0.9484	0.3766
7	22	2.4747	1.0033	0.3830
29	24	2.7238	0.9293	0.3893
33	89	3.0733	0.6950	0.4104
34	103	2.1782	1.2029	0.4145
25	97	2.8959	0.7690	0.4286
30	36	2.8759	0.7964	0.4379
8	34	2.6250	0.9531	0.4410
32	29	3.1796	0.6974	0.4567
9	30	3.0099	0.8643	0.6225
36	32	2.5325	0.6897	0.6386
21	110	3.1939	0.8881	0.8504

Figure 3 Dispersion of the conversion line for individual laboratories, labs no 21, 36, 9 and 40 due to very high or low Sy,x.

The conversion equation

A Linear Mixed Effect (LME) -model was applied to produce the regression line of the 1388 valid samples. ***Multi-step selection of outliers*** (residual standard deviations> 2.58 - see ISO 21187). After application of this procedure 3 times, 65 outliers were eliminated and no further improvement of estimation Sy,x was achieved , consequently no more elimination of outlier data was considered appropriate.

Random effects in the LME were also calculated and 4 labs were found to be over – or underestimating their counts and were consequently deleted.

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By combining 1.474 valid data sets from the first phase (selected with the same statistic model and published in Milk Science Int., 65,3, 2010) and these obtained in the second phase of the project a final conversion equation was computed:

Log 10 cfu /ml = Log 10 IBC/µl * 0.939 + 2.559

Derived from 2.732 samples, with $S_{y:x}$ = 0,282 and applicable from 3 to 70.000 Impulses.

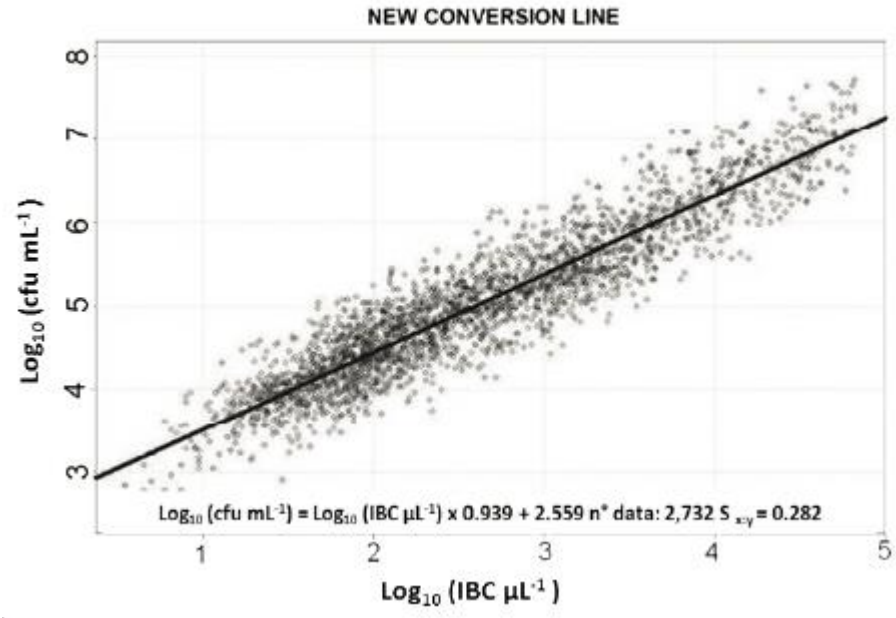

Figure 4 Scatterplot showing trial results and the National Italian conversion equation

Closing remarks

The main negative consequence of the adoption of unique standardized conversion at national level is a bigger uncertainty of accuracy (the real number of CFU´s produced by the bacteria of a milk sample) for someone of the participating labs.

The advantage is a better uniformity (reproducibility) of the results produced by different labs in the country on the same milk sample. A secondary benefit is, the possibility of labs that did not participate in the project to adopt the same conversion line, considering that it is representative of all the cow milk produced in the country.

Read the full trial report on http://www.chiriottieditori.it/ojs/index.php/ijfs/article/view/186

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