

Method Comparison

## Proficiency Tests confirm improved Accuracy and Consistency by Inlabtec Serial Diluter

*"Until we tested it we were not aware of the fact that the Serial Diluter would allow us to achieve such a high consistency with all employees with regard to the z score values!" This statement came after the analysis of the results of interlaboratory tests (proficiency tests) by an ISO/IEC 17025 accredited and GMP compliant Swiss testing laboratory. The test results show a significant improvement in the accuracy and consistency of Total Viable Count (TVC) determinations using the Inlabtec Serial Diluter compared to the test tube technique. All lab team members are now able to deliver microbiological results of the highest reliability and quality, regardless of professional experience and manual skills.*

### Introduction

DIN EN ISO/IEC 17025 accredited test laboratories must regularly participate in proficiency tests. This enables labs to assess their current measured values as well as their laboratory performance over time. A frequent performance parameter of ring tests is the z score, which indicates how many standard deviations a value is from the mean:

$$z = (X - \mu) / \sigma$$

where z is the z-score, X is the value of the measurement,  $\mu$  is the mean of interlaboratory comparisons (reference value), and  $\sigma$  is the standard deviation for the normally distributed measured values.

The evaluation of z values is based on the following criteria:

| Z | ≤ 2 is considered to be satisfactory

2 < | z | < 3 is considered questionable

| Z | ≥ 3 is considered unsatisfactory

The statistical basis for this is that the analysis results are normally distributed, with a probability of 95% within ± two standard deviations and with 99.7% probability within ± three standard deviations.

For the reported proficiency tests the TVC of two reference milk samples was determined twice per year by the pour plate method by a minimum of 3 technicians and the results sent to the test coordinator. A reference milk sample 1 containing approximately ten times less colony forming units was compared to the reference 2. For serial dilutions the test tube technique was used until 2015 and from 2016 onwards, only the Inlabtec Serial Diluter has been used (Figure 1). Both dilution techniques fulfil the ISO Standard 6887-1\* and were performed according to it.



Figure 1: Inlabtec Serial Diluter installed in the clean bench of the ISO/IEC 17025 accredited and GMP compliant Swiss testing laboratory.

## Results

Table 1: Detailed test results of the proficiency tests of the year 2014 - 2016

TEST TUBE TECHNIQUE	Reference Milk 1			Reference Milk 2		
Ring test 1 May 2014	Ref. Value: <b>29'500 CFU</b>			Ref. Value: <b>310'000 CFU</b>		
	Participant	Value	Z-Score	Participant	Value	Z-Score
	TH	26'590	-0.32	TH	262'727	-0.44
	RS	31'090	0.16	RS	324'545	0.13
	CL	24'000	-0.64	CL	264'545	-0.42
	MJ	20'135	-1.19	MJ	199'090	-1.19
Ring test 2 November 2014	Ref. Value: <b>30'359 CFU</b>			Ref. Value: <b>281'838 CFU</b>		
	Participant	Value	Z-Score	Participant	Value	Z-Score
	MJ	27'091	-0.43	MJ	228'182	-0.56
	TH	19'909	-1.28	TH	134'545	-1.65
	RS	33'818	0.41	RS	324'091	0.48
	MA	35'682	0.65	MA	296'364	0.16
	FM	27'909	-0.30	FM	302'273	0.25

TEST TUBE TECHNIQUE	Reference Milk 1			Reference Milk 2		
Ring test 1 May 2015	Ref. Value: <b>22'109 CFU</b>			Ref. Value: <b>225'457 CFU</b>		
	Participant	Value	Z-Score	Participant	Value	Z-Score
	MA	19'909	-0.14	MA	195'909	-0.32
	PL	21'955	-0.01	PL	235'000	0.11
	MJ	19'136	-0.2	MJ	205'909	-0.22
	TH	23'409	0.12	TH	212'727	-0.15
Ring test 2 November 2015	Ref. Value: <b>24'000 CFU</b>			Ref. Value: <b>198'000 CFU</b>		
	Participant	Value	Z-Score	Participant	Value	Z-Score
	MJ	23'591	-0.08	MJ	152'273	-0.66
	PL	22'409	-0.23	PL	188'182	-0.18
	TH	25'773	0.18	TH	212'727	0.21
	MA	21'364	-0.37	MA	201'364	0.05

INLABTEC SERIAL DILUTER	Reference Milk 1			Reference Milk 2		
Ring test 1 May 2016	Ref. Value: <b>16'982 CFU</b>			Ref. Value: <b>208'929 CFU</b>		
	Participant	Value	Z-Score	Participant	Value	Z-Score
	MA	15'600	-0.07	MA	211'000	0.01
	PL	18'300	0.06	PL	210'000	0.00
	TH	16'500	-0.03	TH	225'000	0.06
	LK	17'400	0.02	LK	225'900	0.07
Ring test 2 November 2016	Ref. Value: <b>22'387 CFU</b>			Ref. Value: <b>213'796 CFU</b>		
	Participant	Value	Z-Score	Participant	Value	Z-Score
	PL	22'727	0.01	PL	235'454	0.08
	AA	22'136	-0.01	AA	224'090	0.04
	MA	23'636	0.05	MA	192'726	-0.09

For the proficiency tests of the year 2014 and 2015 the test tube technique was used for serial dilutions for the TVC determination (see table 1). The z scores of the test results obtained by all lab technicians (participants) are  $\leq 1.65$  and therefore considered to be satisfactory. Nevertheless the z scores of the same reference samples obtained by different participants differ in some cases strongly and are with a difference of 2.13 standard deviations questionable (see table 1, November 2014, ring test 2, z scores -1.65/ 0.48).

The z scores for both reference samples obtained by each participant are mostly in a similar range indicating that the participants treated both samples equally. An exception is participant MA who had always a relatively high difference between the two z scores of the two reference samples indicating some possible variation of the working technique with test tubes between the samples (see table 1).

In general the z-scores for the year 2015 are better as in the year 2014 as a result of more experience and training of the participants (see table 1, for example participant TH)

For the proficiency tests during 2016 the Inlabtec Serial Diluter was used. The z scores of all test results obtained by all lab technicians are  $\leq 0.09$ . The maximum z score differences between participants analysing the same reference samples is 0.17 for that the TVCs of the same sample determined by each technician in the lab differ only by maximal 0.17 standard deviations (see table 1).

The z-scores obtained by each participant are for both reference samples of a testing the same range and also below 0.1 indicating that all participants determined TVC equally and with high accuracy when they use the Inlabtec Serial Diluter.

There was also no training phase detected between Ring test 1 and Ring test 2 in the year 2016 as the participants had the same quality of testing from the beginning independent of their professional experiences (see table 1).

## **Discussion & Conclusions**

The results show a clear improvement in the accuracy and consistency of TVC determinations using the Inlabtec Serial Diluter compared to the test tube technique as it was expected from internal studies and as it was reported by other customers in less detailed manners. Compared to the traditional test tube technique, the Inlabtec Serial Diluter automates sample dilution in a single step which integrates precise dispensing and homogeneous mixing. This leads to higher accuracy as well as to a much higher consistency in test results between technicians as shown in table 1.

The effect of manual sample mixing (vortexing) which can lead to unreliable results is completely excluded by using the Inlabtec Serial Diluter due to the standardized and highly efficient automatic sample dilution. This has been shown already by the Inlabtec method comparison "Inhomogeneous dilution poses a potential risk to food quality & safety".

The lab staff concluded that variations in manual vortexing was the major factor for the inconsistency between participants in the test years 2014 and 2015 as the pipettes and culture media used were the same for all tests made. In addition, the automated dilution performed by the Serial Diluter minimizes the potential of human errors and individual differences in microbiological technique.

Using the Inlabtec Serial Diluter, all lab staff are able to deliver microbiological results of the highest reliability and quality, regardless of professional experience and manual skills. In addition, the technicians benefit from a faster, simplified dilution process as well as from less time spent washing, filling, autoclaving or checking test tubes.

From these results the Swiss testing laboratory's enthusiasm for the Inlabtec Serial Diluter is all too understandable.

For further information on the Inlabtec serial dilute please visit [www.inlabtec.com](http://www.inlabtec.com) or contact the company on [info@inlabtec.com](mailto:info@inlabtec.com)

\*: ISO Standard 6887-1 Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions. and were performed according to it.