



NordVal International Certificate

Issued for:	3M™ Petrifilm™ <i>E.coli</i> / Coliform Count Plate
NordVal No:	014
First approval date:	5 May 2003
Renewal date:	1 June 2017
Valid until:	1 June 2019

3M™ Petrifilm™ *E.coli* / Coliform Count Plate

Manufactured by:
3M Health Care,
Microbiology Products,
St. Paul,
Minnesota 55114-1000
USA

Supplied by:
3M France,
Boulevard de l'oise,
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France


The 3M™ Petrifilm™ *E.coli* / Coliform Count Plate has been fully collaboratively validated and corresponds to AOAC 991.14: Coliform and *Escherichia coli* Counts in Foods Dry Rehydratable Film, and to the previously NMKL 147 (1993). The NMKL 147 was withdrawn in 2004, as this NordVal Certificate was issued. The reference methods used in the comparison is AOAC 966.23 and 966.24 - Coliform Group and *Escherichia coli* Microbiological (MPN) Method.

The 3M™ Petrifilm™ *E.coli* / Coliform Count Plate method has also been compared against the ISO 16649-2: Horizontal method for the enumeration of beta-glucuronidase –positive *Escherichia coli* - Part 2: Colony- count technique at 44°C using (-bromo-4-chloro-3-indoyl beta-D-glucuronate in a minor study. In this renewed certificate, this study has been used as an illustration for the relative trueness.

In 2017, the data from the AOAC collaborative study has been recalculated according to the ISO 16140-2: Method Validation – Part 2 - Protocol for the validation of alternative (proprietary) methods against a reference method. The design of the validation study follows AOAC Protocol, Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis, and not the NordVal International Validation Protocol (ISO 16140-2). NordVal International has reviewed the data and found that the 3M™ Petrifilm™ *E.coli* / Coliform Count Plate performs satisfactorily compared to the AOAC reference methods tested for the enumeration of *E.coli* and Coliform count.

Date: 26 May 2017

Yours sincerely



Hilde Skår Norli
Chair of NordVal International



Nina Skall Nielsen
NMKL Secretary General



PRINCIPLE OF THE METHOD

The 3M Petrifilm *E. coli* / Coliform Count Plate is a sample-ready-culture-medium system, which contains Violet red bile nutrients, a cold-water-soluble gelling agent, a tetrazolium indicator that facilitates colony enumeration and an indicator of glucuronidase activity BCIG.

E. coli: Count all blue colonies with and without gas, after 48 hours of incubation at 37°C.

Coliforms: Count red colonies with gas and all blue colonies with or without gas, after 24 hours of incubation at 37°C.

FIELD OF APPLICATION

The method has been tested on foods.

HISTORY

In 1990, the 3MTMPetrifilmTM *E.coli* / Coliform Count Plate was collaboratively studied and compared against the AOAC most probable number methods. The method was accepted as the AOAC Official Method 991.14: Coliform and *Escherichia coli* Counts in Foods Dry Rehydratable Film. The collaborative validation is published in J.AOAC **74**, 635 (1991).

In 1997, a selectivity study of the method for *E.coli* was carried out by ADRIA, Normandy.

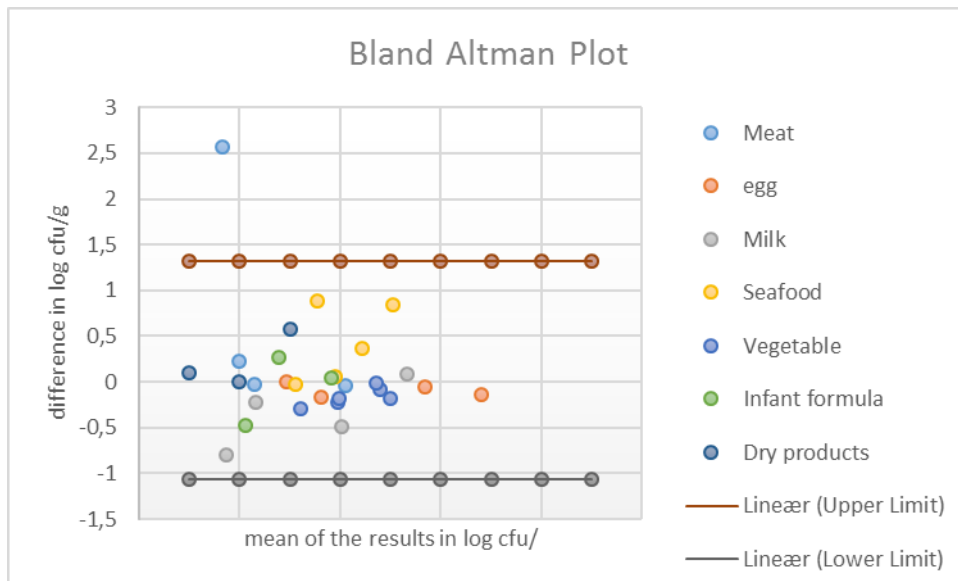
In 2005, a comparison study between the 3MTMPetrifilmTM *E.coli* / Coliform Count Plate and ISO 16649-2 were carried out by ADRIA, Quimper.

In 2017, the results of the collaborative study was recalculated according to the ISO 16140-2:2016.

COMPARISON STUDY

Relative trueness study

In 2005, 41 food samples belonging to different categories; vegetables, egg products, seafood and fish, dry products and infant formula, were analysed in duplicates using 3MTMPetrifilmTM *E.coli* / Coliform Count Plate and ISO 16649-2. The difference between the replicates are plotted in a Bland Altman Plot, using the average of the difference for all categories ± 2 times the standard deviation of differences as the upper and lower limit. It will be expected that no more than 1 in 20 data values will lie outside the 95% confidence levels (upper limit and lower limits). The results are shown below.



Selectivity

The selectivity of the method was studied by by ADRIA Normandy in 1997. Twenty strains of *E. coli* were tested at 37°C, whereof 19 gave blue colonies. One strain (isolated from cheese) gave red colonies. It gave also red colony on PTX medium, and on another validated chromogenic media. 10 non *E. coli* coliform colonies were developing red colonies on the plate. Two strains of *Enterocococci* and one strain of *Staphylococcus* were not able to grow on the plate.

COLLABORATIVE (INTERLABORATORY) STUDY RESULTS

A full collaborative study has been carried out of the 3M™ Petrifilm™ *E. coli* / Coliform Count Plate. Fourteen laboratories participated. Three coliform and *E. coli* levels in 6 samples of 4 product types (flour, nuts, cheese, and beef with gravy) and in 3 samples of 2 product types (mushroom and raw turkey) were tested in duplicates by the participants. Some of the matrices are studied in 2 parts. In the first study, 6 foods were analysed by the participating laboratories. However, as the difference between the low and high levels of the organisms in some of the products were too low, i.e. below the 100-fold difference required, a second study was carried out.

ACCURACY PROFILE STUDY

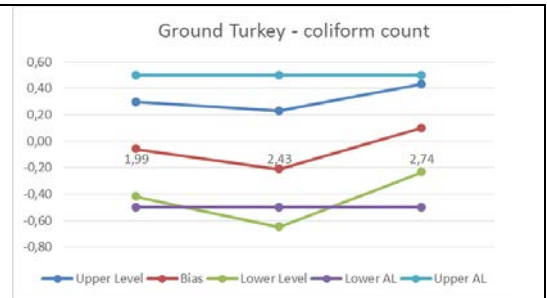
The accuracy profile study is a comparative study between the results obtained by the reference and the results of the alternative method. Six food categories were tested in this collaborative study, and the accuracy profile is given for each category.

TOTAL COLIFORM

The results of Total Coliform for the different matrices are given below.

Table 1: Ground Turkey: Valid results of total coliform (in log cfu/g)

MPN		MPN		Bias	Upper level	Lower level	± AL
Mean	S _R	Mean	S _R				
1.93	0.26	1.99	0.34	-0.06	0.30	-0.42	0.50
2.22	0.32	2.43	0.46	-0.21	0.23	-0.65*	0.50
2.84	0.24	2.74	0.40	0.10	0.43	-0.23	0.50



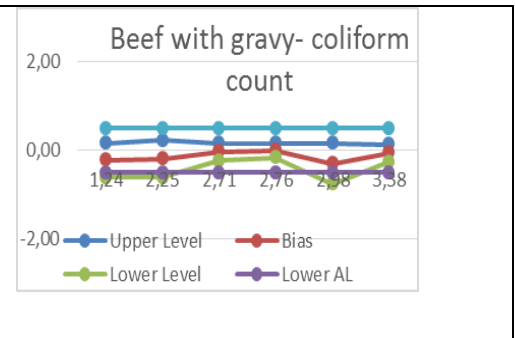
For explanation of AL see below.

* is below AL of -0.5, however, will be within the new AL of 3.3 • Pooled S_R of 3.3 • 0.406 = 1.3

S_R is the standard deviation of the reproducibility including results from all participating laboratories. The standard deviations of the repeatability, S_r, for the parallels analysed (which are smaller than the S_R) are not reproduced in the tables.

Table 2: Beef with gravy: Valid results of total coliform (in log cfu/g)

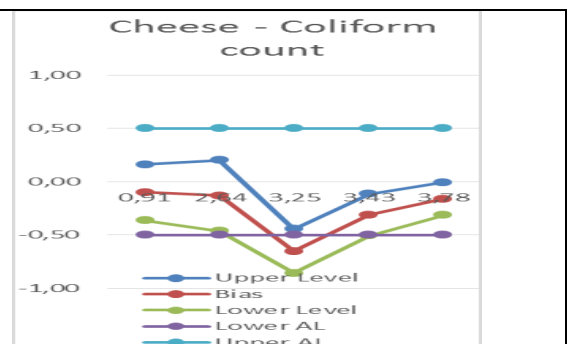
Total coliform		MPN		Bias	Upper level	Lower level	± AL
Mean	S _R	Mean	S _R				
1.02	0.28	1.24	0.39	-0.22	0.17	-0.61*	0.5
2.06	0.3	2.25	0.43	-0.19	0.22	-0.60*	0.5
2.68	0.14	2.71	0.33	-0.03	0.16	-0.22	0.5
2.76	0.12	2.76	0.41	0.00	0.17	-0.17	0.5
2.68	0.33	2.98	0.38	-0.30	0.16	-0.76*	0.5
3.32	0.14	3.38	0.29	-0.06	0.13	-0.25	0.5



* is below AL of -0.5, however, will be within the new AL of 3.3 • Pooled S_R = 3.3 • 0.37 = 1.2

Table 3: Cheese: Valid results of total coliform (in log cfu/g)

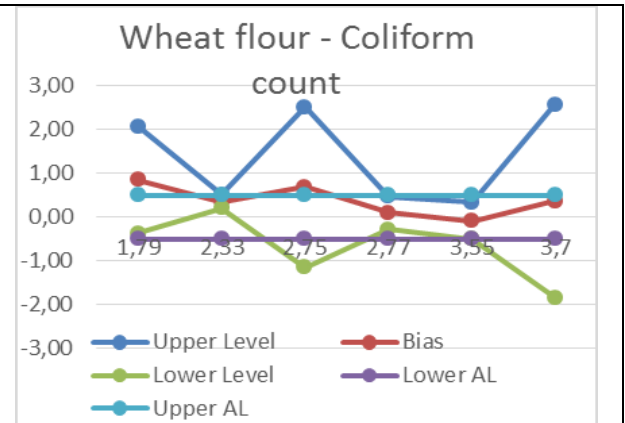
Total coliform		MPN		Bias	Upper level	Lower level	± AL
Mean	S _R	Mean	S _R				
0.81	0.19	0.91	0.32	-0.10	0.16	-0.36	0.5
2.51	0.24	2.64	0.36	-0.13	0.20	-0.46	0.5
2.60	0.15	3.25	0.42	-0.65	-0.44	-0.86*	0.5
3.12	0.16	3.43	0.59	-0.31	-0.09	-0.53	0.5
3.62	0.11	3.78	0.85	0.16	-0.01	-0.31	0.5



* is below AL of -0.5, however, will be within the new AL of 3.3 • pooled S_R of 3.3 • 0.54 = 1.8

Table 4: Flour: Valid results of total coliform (in log cfu/g)

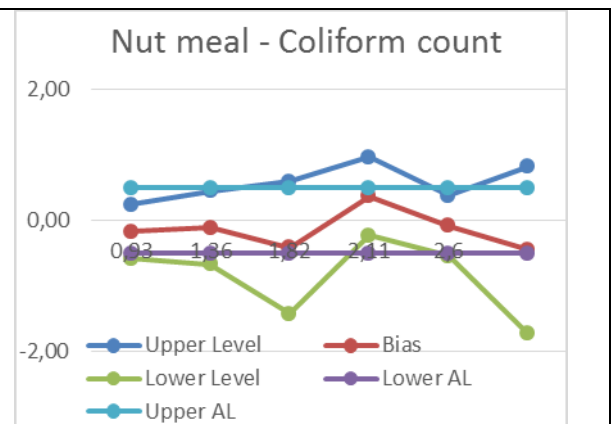
Total coliform		MPN		Bias	Upper level	Lower level	± AL
Mean	S _R	Mean	S _R				
2.64	0.88*	1.79	0.99*	0.85	2.07*	-0.37	0.5
2.68	0.11	2.33	0.53	0.35	0.50	0.20	0.5
3.43	1.32*	2.75	1.10*	0.68	2.51*	-1.15*	0.5
2.86	0.27	2.77	0.53	0.09	0.47	-0.29	0.5
3.46	0.30	3.55	0.51	-0.09	0.33	-0.51	0.5
4.06	1.58*	3.7	1.30*	0.36	2.57*	-1.85*	0.5



For 3 of the 6 samples, the standard deviation, S_R, are not considered satisfactory for neither of the methods. The AL of 3.3•pooled S_R = 3.3 • 0.71 = 2.3. For the levels marked, the upper levels exceeds the new AL, and the method cannot be considered equivalent.

 Table 5: Nut meal: Valid results of total coliform (in log cfu/g)

Total coliform		MPN		Bias	Upper level	Lower level	± AL
Mean	S _R	Mean	S _R				
0.76	0.3	0.93	0.49	-0.17	0.24	-0.58*	0.5
1.25	0.4	1.36	0.47	-0.11	0.45	-0.67*	0.5
1.4	0.73	1.82	0.68	-0.42	0.59	-1.43*	0.5
2.48	0.43	2.11	0.44	0.37	0.97*	-0.23	0.5
2.52	0.33	2.6	0.58	-0.08	0.38	-0.54	0.5
2.36	0.92	2.81	1.0	-0.45	0.82*	-1.72*	0.5



* is outside AL of ±0.5, however, will be within the new AL of 3.3• PooledS_R = 3.3• 0.64 = 2.1

Whenever no biases exist, the results would be on y=0. In the figures above, the acceptability limits (AL) are represented by the purple and the light blue lines. The levels where the results might be expected to vary between (upper and lower levels) are given as green and blue lines. The bias (the difference obtained by the results obtained by the alternative method and the reference method) are given as red lines.

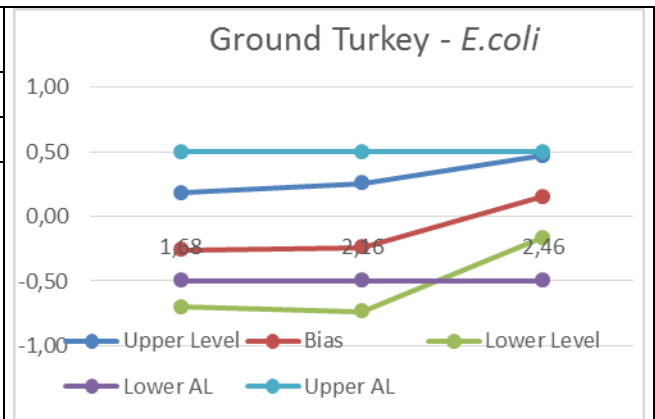
When the upper level (blue line) is below the upper AL (light blue line), and the lower level (green line) is above the lower AL (purple line), the alternative method is accepted as being equivalent to the reference method. If any of the upper or lower levels exceeds the AL and the standard deviation of the reference method, S_R, is above 0.125 log cfu/g, a new AL is calculated as 3.3• pooled S_R. When the AL is adjusted based on the pooled S_R of the reference method, all the results fall, except for flour, are within the acceptability limit.

E.COLI

The results of *E.coli* for the different matrices are given below.

Table 6: Ground Turkey: Valid results of *E.coli* (in log cfu/g)

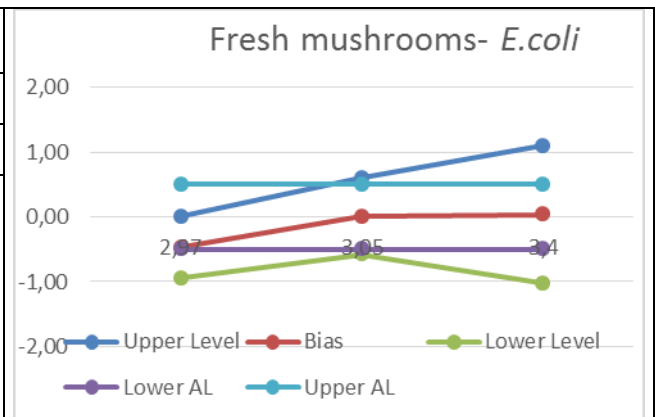
E.coli		MPN		Bias	Upper level	Lower level	± AL
Mean	S _R	Mean	S _R				
1.42	0.32	1.68	0.29	-0.26	0.18	-0.70*	0.5
1.92	0.36	2.16	0.48	-0.24	0.26	-0.74*	0.5
2.61	0.23	2.46	0.5	0.15	0.47	-0.17	0.5



* is below AL of -0.5, however, will be within the new AL of $3.3 \cdot \text{Pooled } S_R = 3.3 \cdot 0.43 = 1.4$

Table 7: Fresh mushrooms: Valid results of *E.coli* (in log cfu/g)

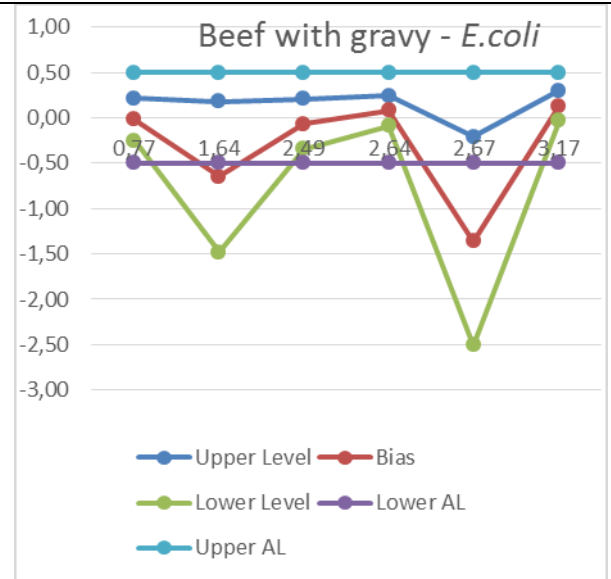
E.coli		MPN		Bias	Upper level	Lower level	± AL
Mean	S _R	Mean	S _R				
2.5	0.34	2.97	0.72	-0.47	0.00	-0.94*	0.5
3.06	0.42	3.05	0.57	0.01	0.60*	-0.58*	0.5
3.44	0.76	3.4	0.89	0.04	1.10*	-1.02*	0.5



* is outside AL of ±0.5, however, will be within the new AL of $3.3 \cdot \text{Pooled } S_R = 3.3 \cdot 0.74 = 2.4$

Table 8: Beef with gravy: Valid results of *E.coli* (in log cfu/g)

E.coli		MPN		Bias	Upper level	Lower level	± AL
Mean	S _R	Mean	S _R				
0.75	0.17	0.77	0.43	-0.02	0.22	-0.26	-0.5
0.99	0.6	1.64	0.30	-0.65	0.18	-1.48*	-0.5
2.42	0.2	2.49	0.51	-0.07	0.21	-0.35	-0.5
2.72	0.12	2.64	0.57	0.08	0.25	-0.09	-0.5
1.31	0.83	2.67	0.34	-1.36	-0.21	-2.51**	-0.5
3.30	0.12	3.17	0.60	0.13	0.30	-0.04	-0.5

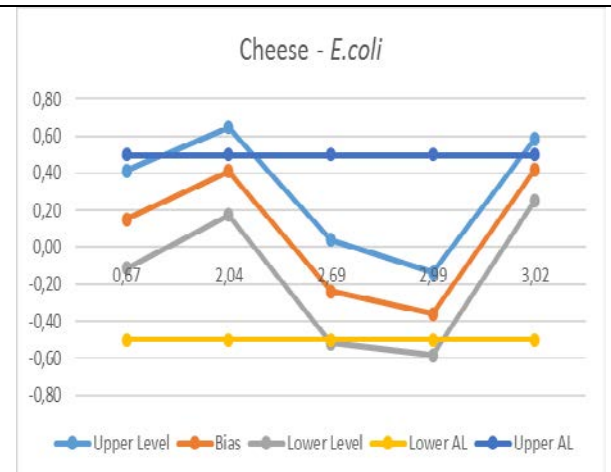


* is below AL of -0.5 log cfu/g, however, will be within the new AL of 3.3 • Pooled S_R = 3.3 • 0.47 = 1.6

** is below the new AL of -1.6 (log cfu/g); and hence not satisfactory

 Table 9: Cheese: Valid results of *E.coli* (in log cfu/g)

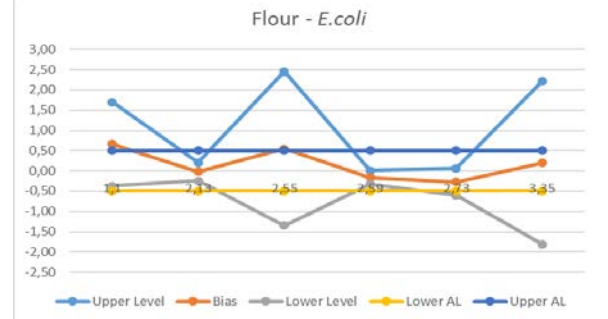
E.coli		MPN		Bias	Upper level	Lower level	± AL
Mean	S _R	Mean	S _R				
0.82	0.19	0.67	0.21	0.15	0.41	-0.11	0.5
2.45	0.17	2.04	0.23	0.41	0.65*	0.17	0.5
2.45	0.2	2.69	0.34	-0.24	0.04	-0.52	0.5
2.63	0.16	2.99	0.57	-0.36	-0.14	-0.58*	0.5
3.44	0.12	3.02	0.30	0.42	0.59*	0.25	0.5



* exceeds AL of ±0.5, however, will be within the new AL of 3.3 • Pooled S_R = 3.3 • 0.35 = 1.2

Table 10: Flour: Valid results of *E.coli* (in log cfu/g)

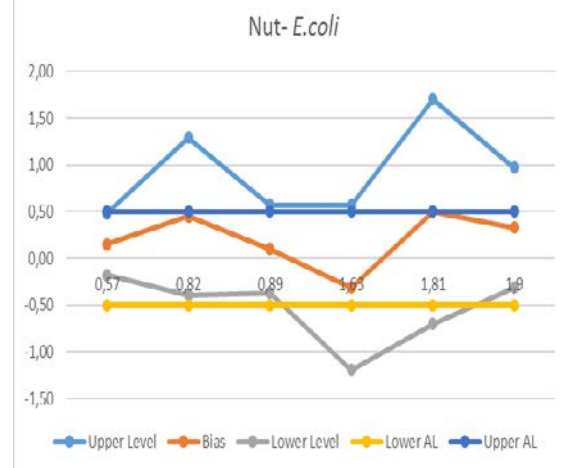
E.coli		MPN		Bias	Upper level	Lower level	± AL
Mean	S _R	Mean	S _R				
1.76	0.74	1.1	0.76	0.66	1.69*	-0.37	0.5
2.11	0.16	2.13	0.77	-0.02	0.20	-0.24	0.5
3.1	1.4	2.55	1.1	0.55	2.45*	-1.35*	0.5
2.43	0.12	2.59	0.82	-0.16	0.01	-0.33	0.5
2.46	0.24	2.73	1.0	-0.27	0.06	-0.60*	0.5
3.55	1.4	3.35	1.3	0.2	2.21*	-1.81*	0.5



* exceeds AL of ±0.5, however, but is within the new AL of 3.3 • Pooled S_R = 3.3 • 0.99 = 3.3

 Table 11: Nut meal: Valid results of *E.coli* (in log cfu/g)

E.coli		MPN		Bias	Upper level	Lower level	± AL
Mean	S _R	Mean	S _R				
0.72	0.24	0.57	0.59	0.15	0.48	-0.18	0.5
1.27	0.61	0.82	0.57	0.45	1.29*	-0.39	0.5
0.99	0.34	0.89	0.46	0.1	0.57*	-0.37	0.5
1.32	0.63	1.63	1.12	-0.31	0.57*	-1.19*	0.5
2.31	0.86	1.81	0.75	0.5	1.70*	-0.70*	0.5
2.23	0.46	1.90	0.61	0.33	0.97*	-0.31	0.5



* exceeds AL of ±0.5, however, but is within the new AL of 3.3 • Pooled S_R = 3.3 • 0.72 = 2.4

Whenever no biases exist, the results would be on $y=0$. When the upper level is below the upper AL and the lower level is above the lower AL, the alternative method is accepted as being equivalent to the reference method. If any of the upper or lower levels exceeds the AL, and the standard deviation of the reference method, S_R, is above 0.125 log cfu/g, then a new AL is calculated as 3.3 • Pooled S_R.

In this study, the levels fall within the new AL, and hence the alternative method is accepted as being equivalent to the reference method.