**NMKL - NordVal International**
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# NordVal International Certificate

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|---|---|
| Issued for: | Compact Dry CF Method for the Enumeration of Total Coliforms |
| NordVal No: | 035 |
| First approval date: | 01 December 2008 |
| Renewal date: | 22 November 2022 |
| Valid until: | 01 December 2024 |

## Compact Dry CF

**Manufactured and supplied by:**

Shimadzu Diagnostics Corporation,
20th Floor Ueno Frontier Tower,
3-24-6 Ueno, Taito-ku, Tokyo,
110-8736 JAPAN

The principle of this method is growth of dedicated bacteria on specific chromogen media.

The performance of this method has been compared to the reference method ISO 4832:2006: “Microbiology of foods and animal feeding stuffs. Horizontal method for the enumeration of coliforms. Colony-count technique”.

The validation studies have been conducted by Campden BRI, UK, according to ISO 16140-2:2016 and NordVal International Protocol 1.

NordVal International concludes that Compact Dry TC provides equivalent results to ISO 4832:2006 for a broad range of foods. The production of Compact Dry TC is certified according to ISO 9001 and ISO 13485.

Date: 22 November 2022

Yours sincerely,

Hilde Skår Norli
Chair of NordVal International

Eystein Oveland
NMKL Executive Director

## PRINCIPLE OF THE METHOD

Compact Dry CF is a ready-to-use chromogenic plate containing for the enumeration of coliforms. Pre-treat the samples according to ISO 6687 or NMKL 91. An aliquot of 1ml of an appropriate dilution is plated onto Compact Dry CF plate. The plate is inverted and incubated at 37 ± 1°C and colonies (blue/blue green) were counted after 24 ± 2h.

## FIELD OF APPLICATION

The method has been tested on enumeration of total coliforms in a broad range of foods.

## HISTORY

In 2007, the method was validated according to the ISO 16140:2003. Every two years until 2018 the method has been renewed without any additional studies.

In 2018 a renewal study was performed to comply with the requirements for relative trueness and accuracy profile in the new standard ISO 16140-2:2016. As the design of the Inter-laboratory study (ILS) is the same for the 2003 and 2016 versions of ISO16140, the data from the ILS data of 2007 are re-evaluated using the new statistical approach outlined in ISO16140-2:2016.

## COMPARISON STUDY

### Relative trueness study

The trueness study is a comparative study between results obtained by the reference method and the results of the alternative method. Different categories, types and items were tested as shown in Table 1 below.

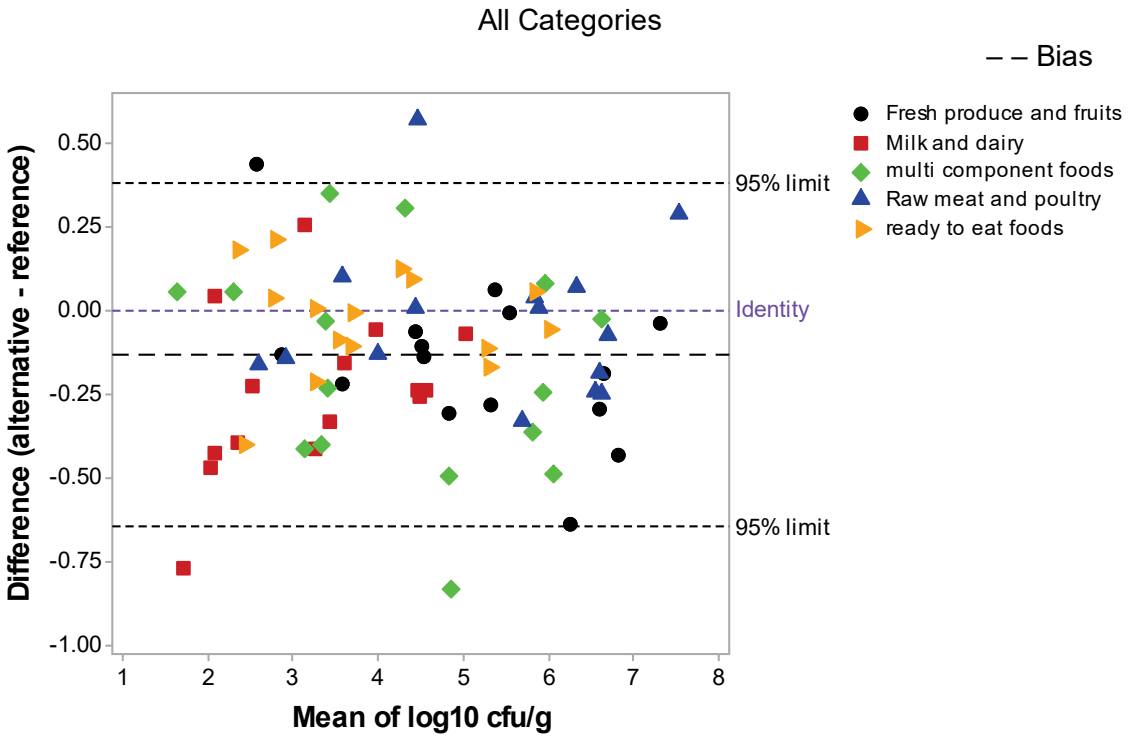
*Table 1. Categories and types tested*

| Category | Types | No. of samples |
|---|---|---|
| Heat processed milk and dairy products | Dry milk product e.g. milk powder, powder for milk based desserts, dried infant formula | 5 |
| | Dairy products e.g. ice-cream, yogurts, cream, hard cheese, soft cheese, raw milk cheese | 5 |
| | Pasteurised milk products e.g. skimmed, semi-skimmed, full fat and flavoured milks | 5 |
| Fresh produce and fruits | Cut ready to eat fruit e.g. fruit mixes, fruit juices | 5 |
| | Cut ready to eat vegetables e.g. Bagged pre-cut salads and shredded carrot, cabbage, vegetable juices | 5 |
| | Leafy greens/Sprouts e.g. soy, mung, alfalfa, | 5 |
| Raw poultry and meats (Combined category raw/ RTC meats and poultry) | Fresh poultry cuts e.g. turkey breast, turkey fillet | 5 |
| | Fresh mince e.g. lamb, beef, pork | 5 |
| | Processed ready to cook e.g. frozen patties, marinated kebabs, seasoned chicken breasts | 5 |
| Ready to eat foods (Combined category RTE/RTRH meats and poultry) | Ready to eat poultry e.g. turkey fillet, chicken sausage, pate | 5 |
| | Cooked fish products e.g. prawns, terrine, pate, smoked fish | 5 |
| | Cooked meat e.g. ham, salami, pate, corned beef | 5 |
| Multi component foods or meal components | Ready to re-heat refrigerated food e.g. cooked chilled foods, rice and pasta, products | 5 |
| | Ready to re-heat food frozen e.g. fries, pizza | 5 |
| | Composite foods with substantial raw ingredients e.g. .pasta salads, sandwiches, deli-salads | 5 |

The relative trueness is illustrated by the use of a Bland-Altman plot, i.e. the difference (bias) between paired samples analysed with the reference method and the alternative method respectively, plotted against the mean values obtained by the reference method. In the plot, Upper and Lower limits are included as the bias ± 2 times the standard deviation of the bias.

The Bland-Altman Plot in Figure 1, illustrates the difference obtained in the enumeration of *Enterobacteriaceae* in foods by the alternative and the reference method, respectively.

*Figure 1. Bland-Altman Plot of the food categories tested*




For 'All Categories' there are four in 75 values which lie outside the CLs. The results obtained are in accordance with the expectations of no more than one of 20 results outside the CLs.

For the four points which were outside of the CLs, there were no identifiable trends in the data and they covered 4 different food categories, 3 different inoculated strains and a range of stresses

## Accuracy profile

The accuracy profile study is a comparative study between the results obtained by the reference method and the results of the alternative method. Each item used were artificially contaminated obtaining three target levels; low ($10^2$ cfu/g), medium ($10^4$ cfu/g) and high ($10^6$ cfu/g). Five test portions of each level of each item were analysed, resulting in 150 samples. The tested categories, types, items and inoculated strains are provided in the Table 2.

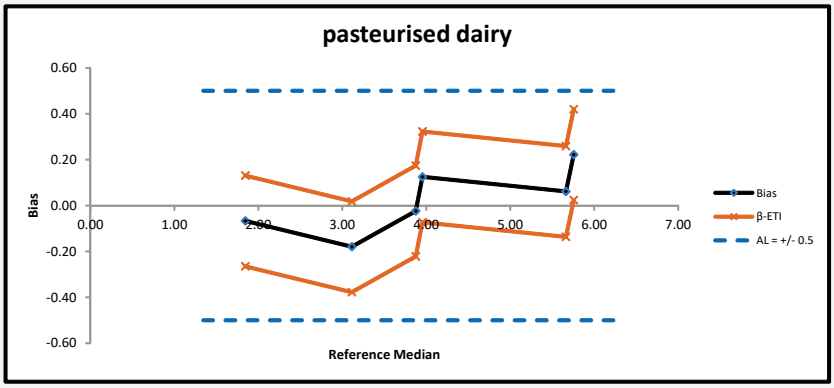
*Table 2. Categories, types and food items*

| Category | Types | Strain | Item |
|---|---|---|---|
| Dairy products | Pasteurised dairy products | *E. coli* CRA 1476 from dried milk | Pasteurised cream |
| | | *Enterobacter agglomerans* CRA 5613 from milk powder | Cream cheese |
| Fruits and vegetables | Fresh produce | *E.hermanii* CRA 7477 from sesame seeds | Ready to cook Vegetable preparation |
| | | *Citrobacter amalonaticus* CRA 7458 from beansprouts | Vegetable juice |
| Raw poultry and meats (Combined category raw/ RTC meats and poultry) | Fresh meat | *Enterobacter aerogenes* NCTC 10006 | Pork mince |
| | | *Citrobacter freundii* NCTC 9750 | Raw bacon |
| Ready to eat foods (Combined category RTE/RTRH meats and poultry) | Cooked fish products e.g. prawns | *E.coli* CRA 2003 from fish | Fresh prawns |
| | | *Klebsiella oxytoca* ATCC 15926 | Fish pate |
| Multi component foods | Composite foods with raw ingredients | *Enterobacter agglomerans* CRA 5513 from skimmed milk powder | Sandwiches |
| | | *E. adecarboxylata* CRA 5501 from skimmed milk powder | Cooked chilled rice |

The statistical results and the accuracy profiles are provided in the Figures 2 to 6.

*Figure 2. Dairy Products*

| (Food) Category | dairy |
|---|---|
| (Food) Type | pasteurised dairy |

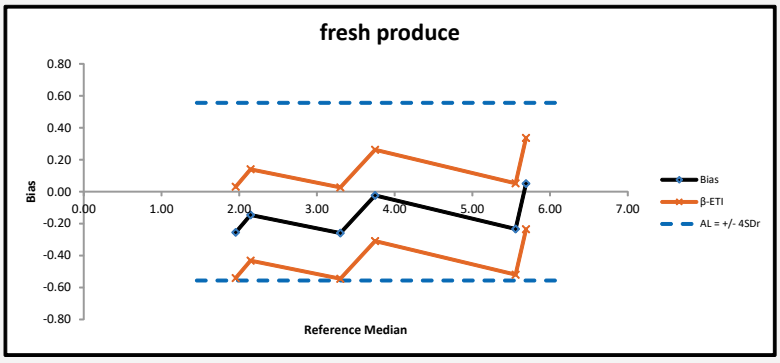



| Sample Name | Reference central value | Bias | Lower β-ETI | Upper β-ETI | β-ETI compared to AL=±0.5 Acceptable | β-ETI compared to final AL Acceptable |
|---|---|---|---|---|---|---|
| 142 21 248 188 53 | 1.85 | -0.067 | -0.265 | 0.131 | YES | YES |
| 107 45 75 342 322 | 3.11 | -0.179 | -0.377 | 0.018 | YES | YES |
| 31 10 97 13 273 | 3.88 | -0.024 | -0.222 | 0.174 | YES | YES |
| 314 346 25 63 328 | 3.95 | 0.125 | -0.073 | 0.323 | YES | YES |
| 196 229 57 102 259 | 5.66 | 0.062 | -0.136 | 0.259 | YES | YES |
| 58 141 172 83 109 | 5.76 | 0.222 | 0.024 | 0.420 | YES | YES |

| | Reference method | Alternative method | SD repeatability of reference method <= 0.125 | Final AL |
|---|---|---|---|---|
| SD Repeatability | 0.132 | 0.137 | NO | +/- 0.500 |

*Figure 3. Fruit and vegetable products*

| (Food) Category | fruit and veg |
|---|---|
| (Food) Type | fresh produce |

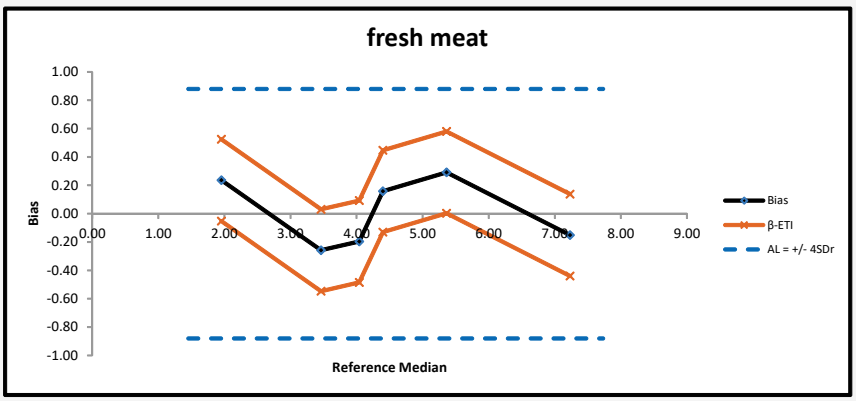



| Sample Name | Reference Central value | Bias | Lower β-ETI | Upper β-ETI | β-ETI compared to AL=±0.5 Acceptable | β-ETI compared to final AL Acceptable |
|---|---|---|---|---|---|---|
| 8 17 327 246 37 | 1.95 | -0.255 | -0.541 | 0.031 | NO | YES |
| 264 252 139 300 294 | 2.15 | -0.146 | -0.432 | 0.140 | YES | YES |
| 312 301 64 267 340 | 3.30 | -0.260 | -0.545 | 0.026 | NO | YES |
| 236 92 223 9 158 | 3.75 | -0.024 | -0.310 | 0.262 | YES | YES |
| 316 86 323 117 203 | 5.56 | -0.234 | -0.520 | 0.052 | NO | YES |
| 74 234 272 34 144 | 5.69 | 0.050 | -0.236 | 0.336 | YES | YES |

| | Reference method | Alternative method | SD repeatability of reference method <= 0.125 | Final AL |
|---|---|---|---|---|
| SD Repeatability | 0.139 | 0.198 | NO | +/- 0.556 |

*Figure 4. Meat and poultry*

| (Food) Category | raw poultry and meat |
|---|---|
| (Food) Type | fresh meat |

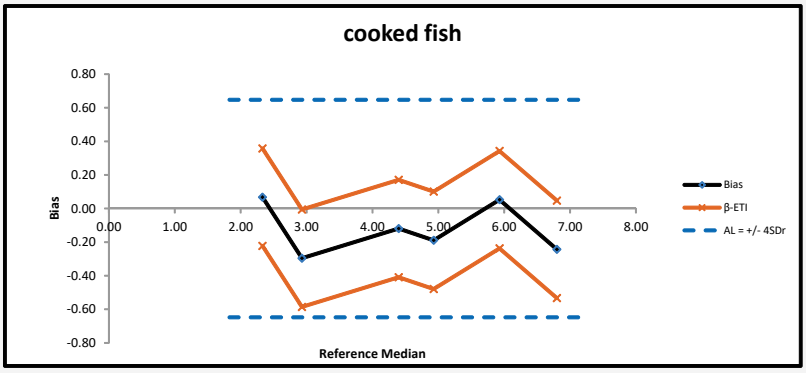



| Sample Name | Reference Central value | Bias | Lower β-ETI | Upper β-ETI | β-ETI compared to AL=±0.5 Acceptable | β-ETI compared to final AL Acceptable |
|---|---|---|---|---|---|---|
| 168 135 283 214 47 | 1.95 | 0.236 | -0.053 | 0.525 | NO | YES |
| 204C 253C 207CC76CC 156C | 3.46 | -0.258 | -0.547 | 0.030 | NO | YES |
| 284E 225E 85E 257E 96E | 4.04 | -0.196 | -0.485 | 0.092 | YES | YES |
| 348 171 65 281 151 | 4.40 | 0.158 | -0.130 | 0.447 | YES | YES |
| 167 36 324 216 133 | 5.36 | 0.291 | 0.003 | 0.580 | NO | YES |
| 165C 69C 310C 118C 349C | 7.23 | -0.151 | -0.440 | 0.137 | YES | YES |

| | Reference method | Alternative method | SD repeatability of reference method <= 0.125 | Final AL |
|---|---|---|---|---|
| SD Repeatability | 0.220 | 0.200 | NO | +/- 0.880 |

*Figure 5. Ready to eat foods*

| (Food) Category | RTE foods |
|---|---|
| (Food) Type | cooked fish |

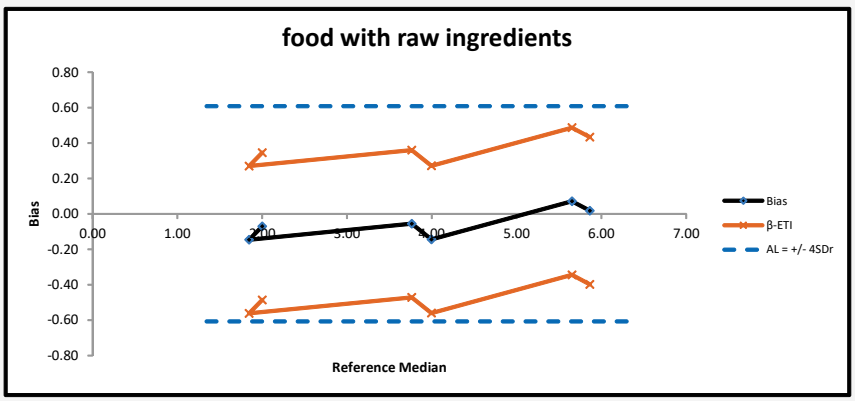



| Sample Name | Reference Central value | Bias | Lower β-ETI | Upper β-ETI | β-ETI compared to AL=±0.5 Acceptable | β-ETI compared to final AL Acceptable |
|---|---|---|---|---|---|---|
| 51 247 218 87 287 | 2.33 | 0.068 | -0.223 | 0.358 | YES | YES |
| 111 155 255 186 202 | 2.93 | -0.296 | -0.586 | -0.006 | NO | YES |
| 289 68 23 309 226 | 4.40 | -0.119 | -0.409 | 0.171 | YES | YES |
| 256 192 295 16 298 | 4.93 | -0.189 | -0.479 | 0.101 | YES | YES |
| 195 261 42 61 320 | 5.93 | 0.053 | -0.237 | 0.343 | YES | YES |
| 319 189 78 82 182 | 6.80 | -0.243 | -0.533 | 0.047 | NO | YES |

| | Reference method | Alternative method | SD repeatability of reference method <= 0.125 | Final AL |
|---|---|---|---|---|
| SD Repeatability | 0.162 | 0.201 | NO | +/- 0.648 |

*Figure 6. Multi component foods:*

| (Food) Category | Mulit component |
|---|---|
| (Food) Type | food with raw ingredients |




| Sample Name | Reference Central value | Bias | Lower β-ETI | Upper β-ETI | β-ETI compared to AL=±0.5 Acceptable | β-ETI compared to final AL Acceptable |
|---|---|---|---|---|---|---|
| 180 304 306 258 55 | 2.00 | -0.071 | -0.486 | 0.345 | YES | YES |
| 329 30 146 275 2422 | 1.85 | -0.146 | -0.562 | 0.270 | NO | YES |
| 242 184 211 334 331 | 3.76 | -0.056 | -0.472 | 0.360 | YES | YES |
| 18 175 1 143 138 | 4.00 | -0.144 | -0.560 | 0.271 | NO | YES |
| 131 59 161 103 209 | 5.65 | 0.071 | -0.345 | 0.487 | YES | YES |
| 245 115 224 249 315 | 5.86 | 0.017 | -0.398 | 0.433 | YES | YES |

| | Reference method | Alternative method | SD repeatability of reference method <= 0.125 | Final AL |
|---|---|---|---|---|
| SD Repeatability | 0.152 | 0.288 | NO | +/- 0.608 |

The observed profiles are within the 0.5 log AL (for dairy products) or the recalculated AL limit calculated according to ISO16140-2:2015 section 6.1.3.3 (other products).

All the accuracy profiles fulfil the performance criteria after the permitted recalculation and the alternative method is accepted as being equivalent to the reference method.

## Selectivity of the method (Inclusivity/ exclusivity)

Inclusivity is the ability of an alternative method to detect the target analyte from a wide range of strains. In the original study, all 33 strains studied revealed typical colonies. Of the 20 inclusivity strains tested in the current study, 18 strains were detected using the alternative and reference methods. Those not detected by the alternative method were *Shimwellia blattae* NCTC 12127 and *Klebsiella rhinoscleromatis* CRA 4272.

Exclusivity is the lack of interference from a relevant range of non-target strains of the alternative method. In the original study, 20 were studied. 10 strains did not grow on the CF medium and 8 were atypical in appearance. Two strains of *Shigella sonnei* appeared typical owing to the presence of galactosidase activity. By comparison, 9 stains failed to grow in VRBA, 5 strains were atypical and 6 were typical in appearance.
In the recent study, 10 non-target strains were tested. Of these strains, three strains (*A.hydrophila* CRA 4111, *A.sobria* CRA 8390 and *S. Fonticola* CRA 4613) were detected by both methods. An additional two strains (*Serratia liquefaciens* CRA 10670 and *Serratia proteamaculans* 1643) were detected by the reference method but not the alternate method.

The alternate method is more selective than the reference method.

## Conclusion of the comparison study

The results of the method comparison study clearly showed that the Compact Dry ETB is equivalent to or better (regarding the selectivity) than the reference method ISO 4832:2006. The lowest validated level is about 2 log cfu/g.

## INTERLABORATORY STUDY

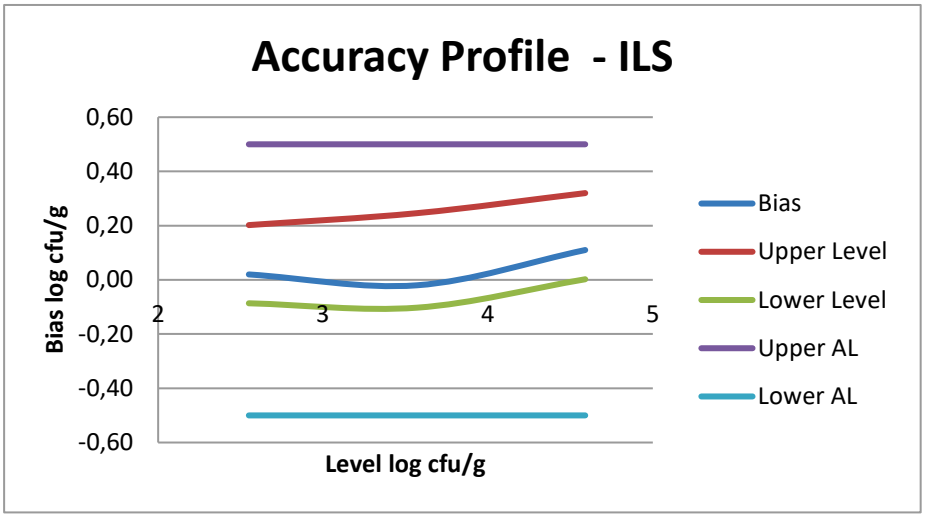
The interlaboratory study was conducted in November 2007. Eleven laboratories analysed samples of pasteurised milk artificially contaminated with defined numbers of *Esherichia coli* and *Enterobacter aerogenes.* The laboratories performed the analyses according to ISO 4832 and Compact Dry CF.

*Table 3. Results (log cfu/g) of the collaborative study*

| | Referance method | | Alternative method | | | Upper | Lower | Upper | Lower |
|---|---|---|---|---|---|---|---|---|---|
| Level | Median | $S_R$ | Alt method | $S_R$ | Bias | Level | Level | AL | AL |
| 1 | 2.53 | 0.16 | 2.55 | 0.13 | 0.02 | 0.20 | -0.09 | 0.50 | -0.50 |
| 2 | 3.59 | 0.11 | 3.57 | 0.19 | -0.02 | 0.25 | -0.10 | 0.50 | -0.50 |
| 3 | 4.48 | 0.075 | 4.59 | 0.15 | 0.11 | 0.32 | 0.00 | 0.50 | -0.50 |

The results show that the bias is small and that the precision is satisfactory. All the results fall within the acceptance levels (AL).

*Figure 7. Accuracy Profile of the interlaboratory study for coliforms*




## CONCLUSION

According to the comparison and the interlaboratory study no substantial differences were found between the Compact Dry CF method and the reference method (ISO 4832:2006) for the enumeration of coliforms.