

NordVal International Certificate

Issued for:	RAPID'<i>E.coli</i> 2 Agar
NordVal No:	020
First approval date:	24 June 2005
Renewal date:	07 February 2024
Valid until:	07 February 2026

RAPID'*E.coli* 2 Agar

Manufactured and supplied by:

Bio-Rad Laboratories,
3 Blvd Raymond Poincaré,
92430 Marnes-la-Coquette, France

fulfils the requirements of the NordVal validation protocol. The reference methods were ISO 4832:2006 for the enumeration of coliforms in foods at 37 °C, and ISO 16649-2:2001 for the enumeration of *E. coli* in foods at 37 °C and 44 °C.

NordVal International has studied the enclosures to the application and evaluated the results obtained in the validations conducted by ISHA and Microsept in accordance with ISO 16140-2:2016. NordVal has concluded that it has been satisfactorily demonstrated that the requirements of the NordVal validation protocol are fulfilled for broad range of foods. The results document no statistical difference in the performances between the RAPID'*E.coli* 2 Agar and the reference methods.

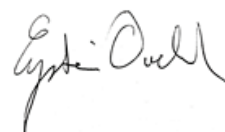
The production of the RAPID'*E.coli* 2 Agar is fulfilling the requirements given in ISO 9001.

Date: 7 February 2024

Yours sincerely,



Hrólfur Sigurðsson
Chair of NordVal International



Eystein Oveland
NMKL Executive Director



PRINCIPLE OF THE METHOD

RAPID'*E.coli* 2 is based on simultaneous detection of two enzyme activities; β -D-Glucuronidase (GLUC) and β -D-Galactosidase (GAL).

RAPID'*E.coli* 2 totally inhibits growth of Gram-positive bacteria and of the principal Gram-negative bacteria other than *Enterobacteriaceae*. *Escherichia coli* specificity is obtained by means of simultaneous detection of β -D-Glucuronidase (GLU) (specific to *E. Coli*) and β -D-Galactosidase (GAL) enzymatic activities.

The medium contains 2 chromogenic substrates:

- √ one substrate specific to GLU, causing pink coloration of colonies positive for this enzyme,
- √ one substrate specific to GAL, causing blue coloration of colonies positive for this enzyme.

Coliforms other than *E. coli* (GAL+/GLUC-) form blue to green colonies, *E. coli* (GLU+/GAL+) form violet to pink colonies.

FIELD OF APPLICATION

The method is applicable for the determination of *E. coli* and total coliforms in a broad range of foods, environmental samples and animal feed. Lowest levels tested were <2 log cfu/g.

HISTORY

June 1995: The RAPID'*E.coli* 2 was approved using the reference method ISO 4832 (1991): Horizontal method for the enumeration of coliforms – colony-count technique.

2003: The certificate was renewed with additional studies from 2001 (Eurofins IPL Nord) using the reference method ISO 16649-2 (2001): Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* -- Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.

2004: Interlaboratory Study was carried out by Eurofins IPL Nord on coliforms using RAPID'*E.coli* 2 and ISO 4832.

2006: ISO 4832 was revised, however it was considered that the validation of alternative methods based on the previous edition were not affected by this revision.

2017: Additional studies were performed by ISHA to assure compliance with the new NMKL protocol and the standard ISO 16140-2:2016.

2024: Additional method extension studies have been performed by Microsept to include the categories environmental samples (year 2022) and animal feed (year 2023) and the validation results were approved by NordVal International.

RESULTS OF THE METHOD COMPARISON STUDY

Selectivity; inclusivity and exclusivity

For coliforms:

Sixty-nine (69) positive strains and thirty (30) negative strains were tested in duplicate by the alternative method. All coliforms strain were cultivated on media and gave characteristic colonies, except for few *Hafnia alvei* strain which give white colonies. These colonies are ONPG negative, that's explained the non-characteristic aspect of the colonies.

Two strains ONPG negative were tested with the reference method (with VRBL incubated at 37°C). One strain gave non characteristics colonies on VRBL and the other didn't grow.

All the negative strains, when they cultivated on the agar media of the alternative method, showed a non-typical aspect, except for a strain of *Shigella sonnei* (β -glucuronidase positive) and two strains of *Salmonella arizonae* (lactose positive).

These three strains were tested with the reference method (with VRBL incubated at 37°C). They gave a typical aspect on the VRBL media. In conclusion, the inclusivity and the exclusivity of the alternative method are satisfactory.

For *E. coli*:

Fifty (50) positive strains and fifty four (54) negative strains were tested in duplicate by the alternative method. All the β -glucuronidase positive *Escherichia coli* strains cultivated on the agar media of the alternative method and gave typical colonies. All the negative strains, when they cultivated on the agar media of the alternative method, showed a non-typical aspect, except for a strain of *Shigella sonnei* (β -glucuronidase positive) and two strains of *Salmonella arizonae* (lactose positive). These three strains were tested with the reference method (pouring in TBX agar media). They also gave a typical aspect on the TBX agar media (blue colonies). These results were observed at both temperatures.

In conclusion, the inclusivity and the exclusivity of the alternative method are satisfactory.

Relative trueness

The relative trueness is the degree of correspondence between the response obtained by the alternative method and the response obtained by reference methods on identical samples. According to ISO 16140-2, five categories should be tested including three different types and at least 15 samples each category. RAPID'*E.coli* 2 is compared against both reference methods, fulfilling the requirement to the number of samples.

The results are illustrated in so called Bland-Altman plot for the difference in the response (bias), including 95% confidence interval of the bias. Bland-Altman difference plots for all categories are shown in **Figures 1-3**, for the different reference methods and temperatures. These plots include the new categories "Environmental samples" and "Animal feed" approved by NordVal 2024. Note that the "Corrected values", and the "Values <4 CFU/Petri dish" are also presented in these plots. The "Corrected values" are results lower or higher than the quantification limits for one method corrected according to the EN ISO 16140-2:2016 requirements. The "Values <4 CFU/Petri dish" are results expressed with less than four colonies per Petri dish for at least one method.

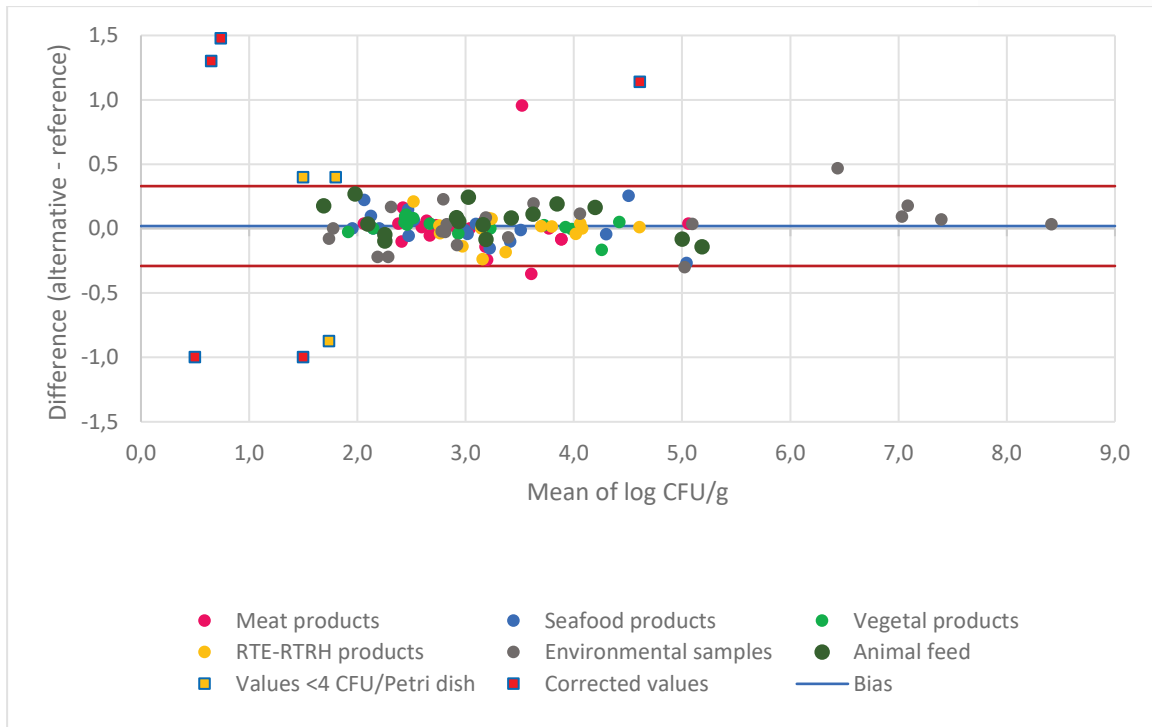


Figure 1. Coliforms: comparison to reference method ISO 4832

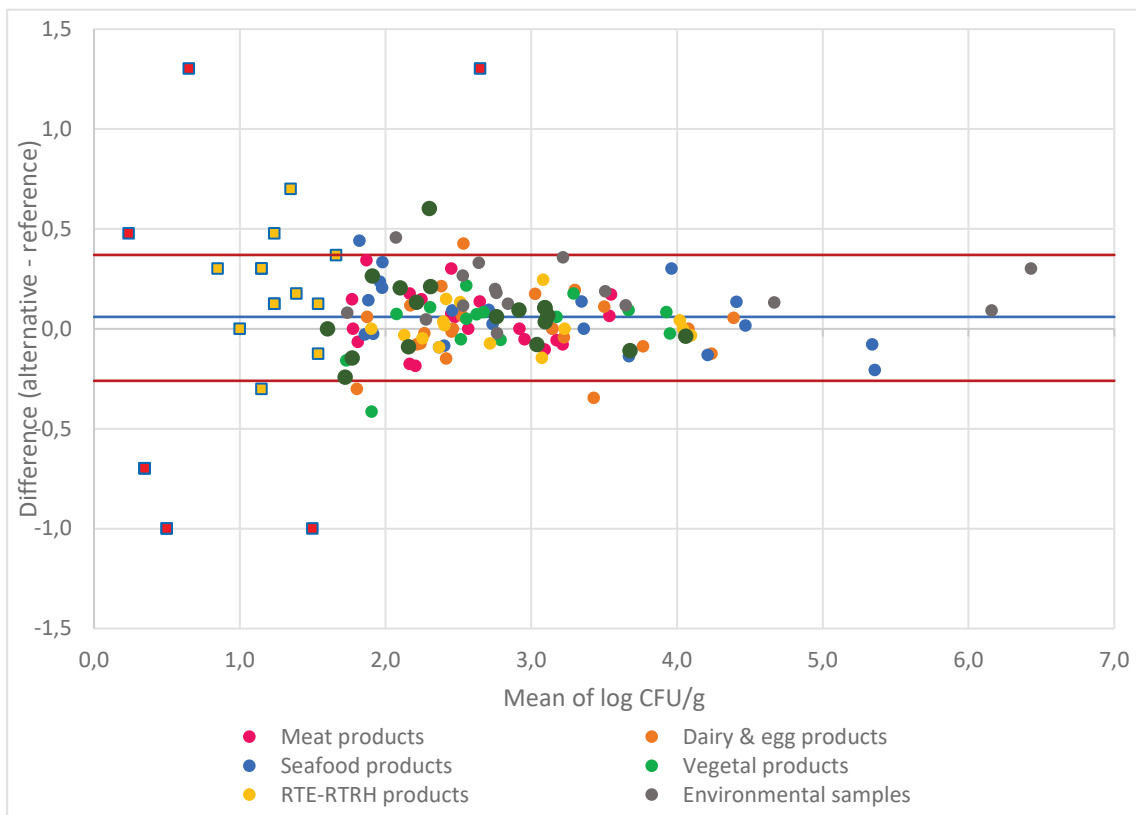


Figure 2. *E. coli*: comparison to reference method ISO 16649-2 at 37 °C

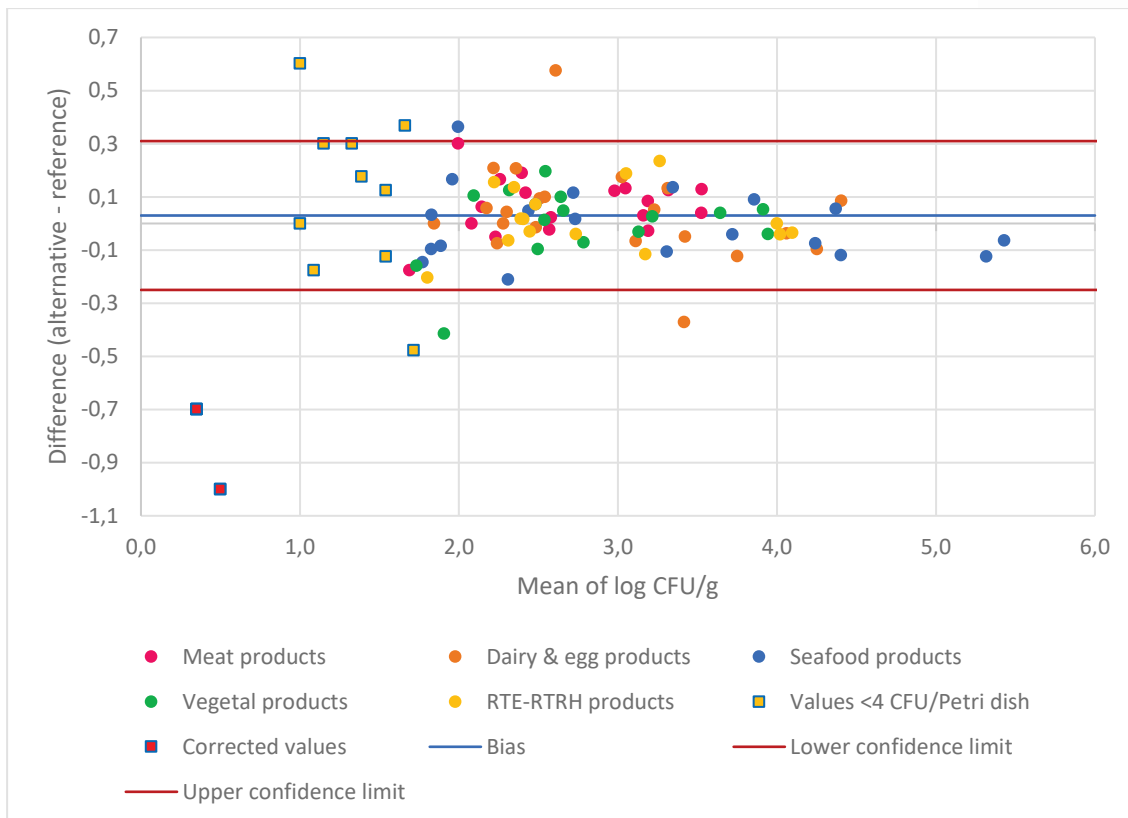


Figure 3. *E. coli*: comparison to reference method ISO 16649-2 at 44 °C

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). In conclusion, the relative trueness of the alternative method is satisfactory compared to reference method ISO 4832, and to ISO 16649-2 regardless of temperature (37 or 44 °C).

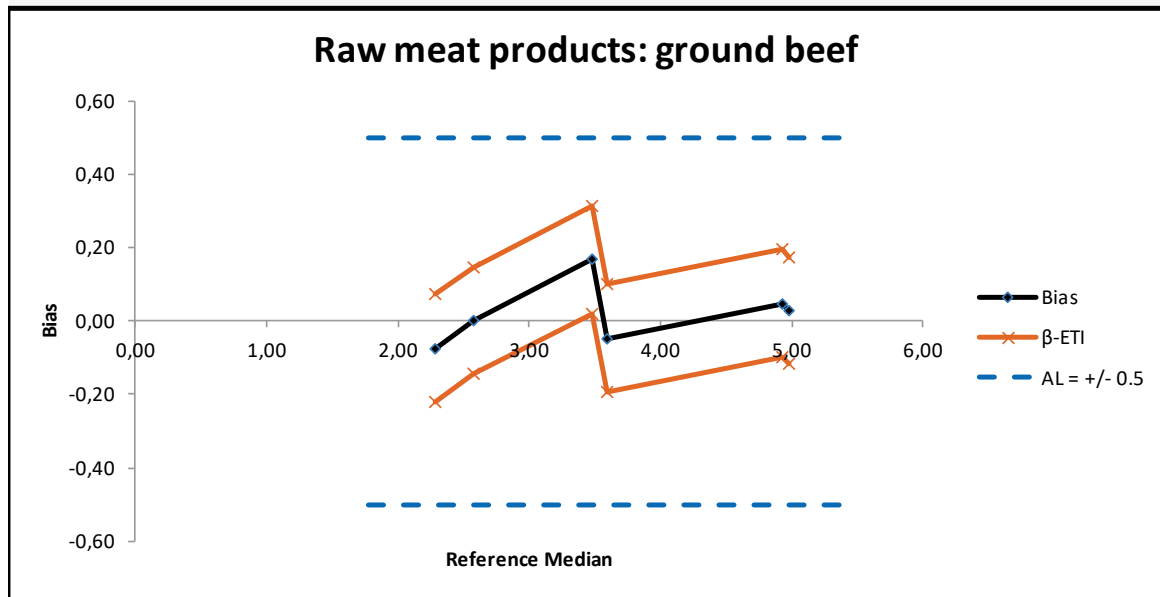
Accuracy profiles

For determination of accuracy profiles, the seven different categories, meat products, dairy products, seafood products, vegetable products, composite foods, environmental samples and animal feed were inoculated at low, medium and high level i.e. 2.5, 3.5 and 5.0 log cfu/g with a coliform strain for comparison with reference method ISO 4832 or with an *E. coli* strain for comparison with reference method ISO 16649-2.

An example of the accuracy profiles for the reference methods against the alternative method is given in **Figure 4**.

See [APPENDIX](#) for all the accuracy profiles.

(Food) Category	Meat products
(Food) Type	Raw meat products: ground beef



Sample Name	Reference central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL= \pm 0.5 Acceptable	β -ETI compared to final AL Acceptable
MML1T1	2,28	-0,075	-0,220	0,071	YES	YES
MML2T1	2,58	0,000	-0,146	0,146	YES	YES
MML1T2	3,48	0,166	0,021	0,312	YES	YES
MML2T2	3,59	-0,047	-0,193	0,099	YES	YES
MML1T3	4,93	0,048	-0,098	0,194	YES	YES
MML2T3	4,97	0,027	-0,119	0,173	YES	YES

	Reference method	Alternative method	SD repeatability of reference method \leq 0.125	Final AL
SD Repeatability	0,210	0,101	NO	+/- 0,500

Figure 4. Coliforms: accuracy profiles ($\beta = 80\%$, AL = 0.5) (reference method, ISO 4832, versus alternative method).

The accuracy profiles of all the categories, with β at 80% and AL (Acceptable Limit) at 0.5, the upper limits of the β -expectation tolerance interval (β -ETI) are inferior to the positive AL of 0.5 and the lower limits of the β -ETI are superior to the negative AL of -0.5. For all samples, the results fall within the AL \pm 0.5.

In conclusion, the accuracy profiles are satisfactory in the range of contamination for coliforms and *E. coli* enumeration.

RESULTS OF THE INTERLABORATORY STUDY

Coliform bacteria

An interlaboratory study was performed in November 2004. The samples were sent to 16 collaborators. The collaborators received 8 samples of pasteurized milk (2 vials per contamination level; 4 contamination levels) to perform the analyses with the reference method ISO 4832 and with the alternative method RAPID'E.Coli 2.

The two strains used for the contamination of the samples were 1) an *Escherichia coli* β -glucuronidase positive isolated from a pastry and 2) an *Enterobacter cloacae* isolated from milk powder.

The results from the interlaboratory test for coliforms at 37°C against the reference method are presented in **Table 1** and **Figure 5**.

Table 1. Results of the interlaboratory test for coliforms at 37°C, reference method was ISO 4832.

Levels	Alternative method			Reference method		
	Low	Medium	High	Low	Medium	High
Target value	1,87	2,93	3,96			
Number of participants	10	10	10	10	10	10
Average for alternative method	1,95	2,98	4,02	1,87	2,93	3,96
Repeatability standard deviation (sr)	0,082	0,037	0,049	0,050	0,046	0,069
Between-labs standard deviation (sL)	0,000	0,065	0,062	0,083	0,082	0,092
Reproducibility standard deviation (sR)	0,082	0,075	0,079	0,097	0,094	0,115
Bias	0,078	0,042	0,056			
Relative Lower TI limit (beta = 80%)	-0,034	-0,064	-0,054			
Relative Upper TI limit (beta = 80%)	0,190	0,149	0,166			
Lower Acceptability Limit	-0,50	-0,50	-0,50			
Upper Acceptability Limit	0,50	0,50	0,50			

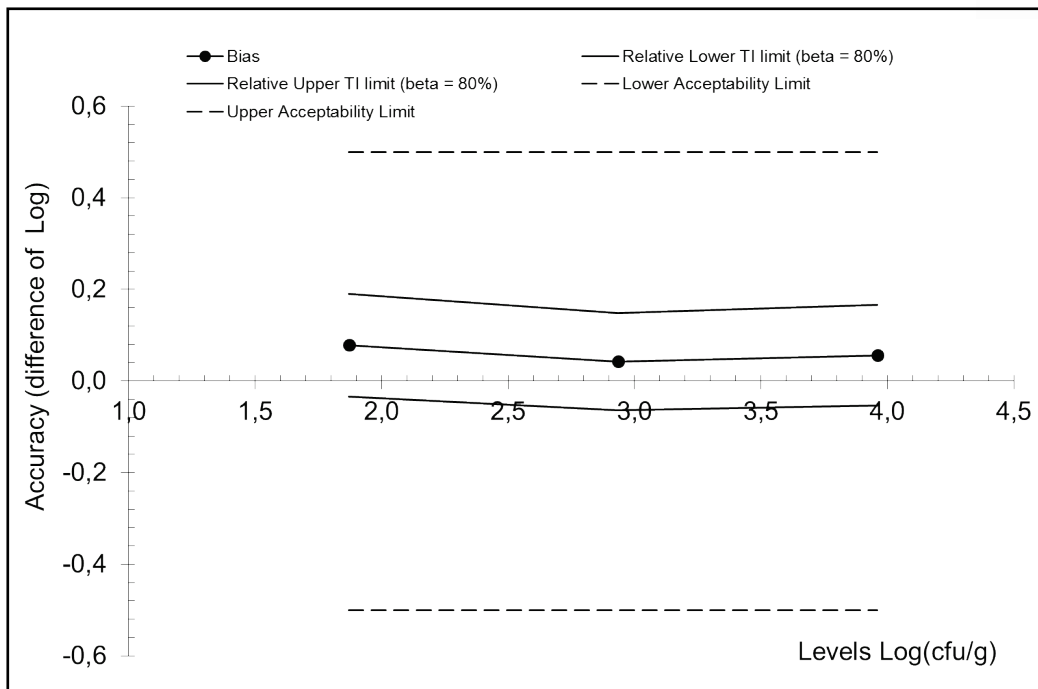


Figure 5. Graphical representation of the accuracy profile of the interlaboratory study for coliform test at 37°C, reference method was ISO 4832.

E. Coli

An interlaboratory study was performed in November 2001. The samples were sent to 16 collaborators. The collaborators received 8 samples of pasteurized milk (2 vials per contamination level; 4 contamination levels) to perform the analyses with the reference method ISO 16649-2 and with the alternative method RAPID'*E. Coli* 2.

The strain used for the contamination of the samples was an *Escherichia coli* β -glucuronidase positive isolated from pastry. Comparison was performed at 37 and 44°C.

The results from the interlaboratory test for *E. coli* at 37°C against the reference method are presented in **Table 2** and **Figure 6** and at 44°C in **Table 3** and **Figure 7**.

Table 2. Results of the interlaboratory study for RAPID'*E. coli* 2 at 37°C, reference method was ISO 16649-2

Levels	Alternative method			Reference method		
	Low	Medium	High	Low	Medium	High
Target value	1,61	2,66	3,72			
Number of participants	10	10	10	10	10	10
Average for alternative method	1,72	2,72	3,75	1,61	2,66	3,72
Repeatability standard deviation (sr)	0,094	0,042	0,061	0,113	0,050	0,093
Between-labs standard deviation (sL)	0,000	0,070	0,000	0,113	0,117	0,082
Reproducibility standard deviation (sR)	0,094	0,082	0,061	0,160	0,127	0,124
Bias	0,119	0,057	0,030			
Relative Lower TI limit (beta = 80%)	-0,009	-0,059	-0,053			
Relative Upper TI limit (beta = 80%)	0,247	0,173	0,112			
Lower Acceptability Limit	-0,50	-0,50	-0,50			
Upper Acceptability Limit	0,50	0,50	0,50			

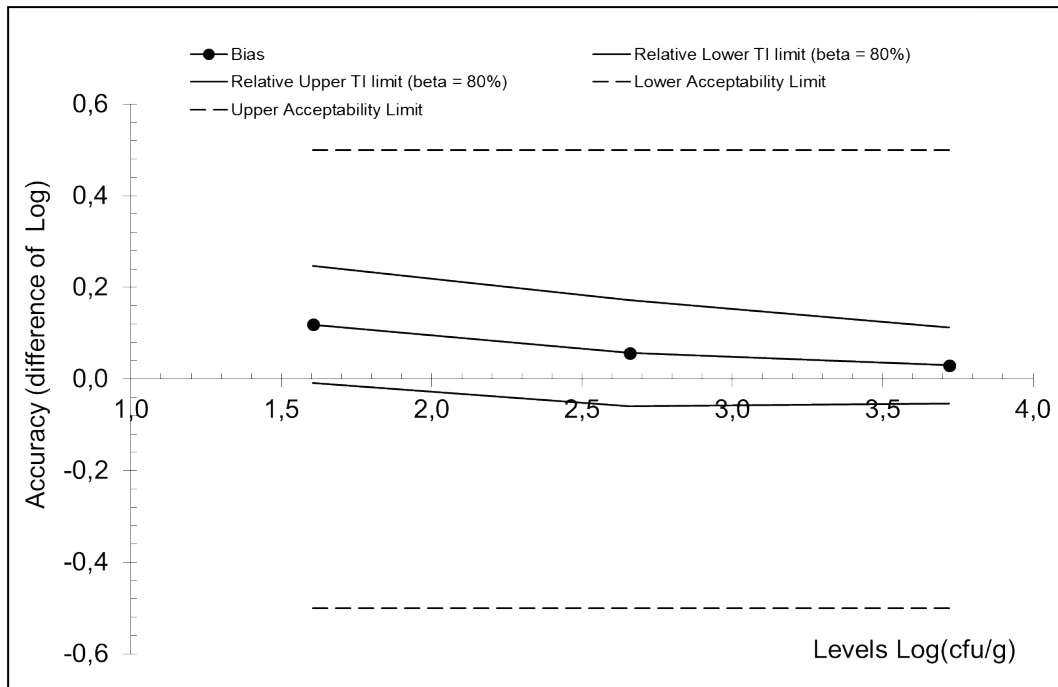


Figure 6. Graphical representation of the accuracy profile of the interlaboratory study for *E. coli* test at 37°C, reference method was ISO 16649-2

Table 3. Results of the interlaboratory study for *E. coli* test at 44°C, reference method was ISO 16649-2

Levels	Alternative method			Reference method		
	Low	Medium	High	Low	Medium	High
Target value	1,49	2,57	3,68			
Number of participants	13	14	14	13	14	14
Average for alternative method	1,60	2,66	3,71	1,49	2,57	3,68
Repeatability standard deviation (sr)	0,067	0,060	0,058	0,173	0,119	0,114
Between-labs standard deviation (sL)	0,106	0,055	0,085	0,000	0,179	0,097
Reproducibility standard deviation (sR)	0,125	0,081	0,103	0,173	0,215	0,150
Bias	0,11	0,09	0,04			
Relative Lower TI limit (beta = 80%)	-0,061	-0,019	-0,106			
Relative Upper TI limit (beta = 80%)	0,285	0,201	0,176			
Lower Acceptability Limit	-0,50	-0,50	-0,50			
Upper Acceptability Limit	0,50	0,50	0,50			

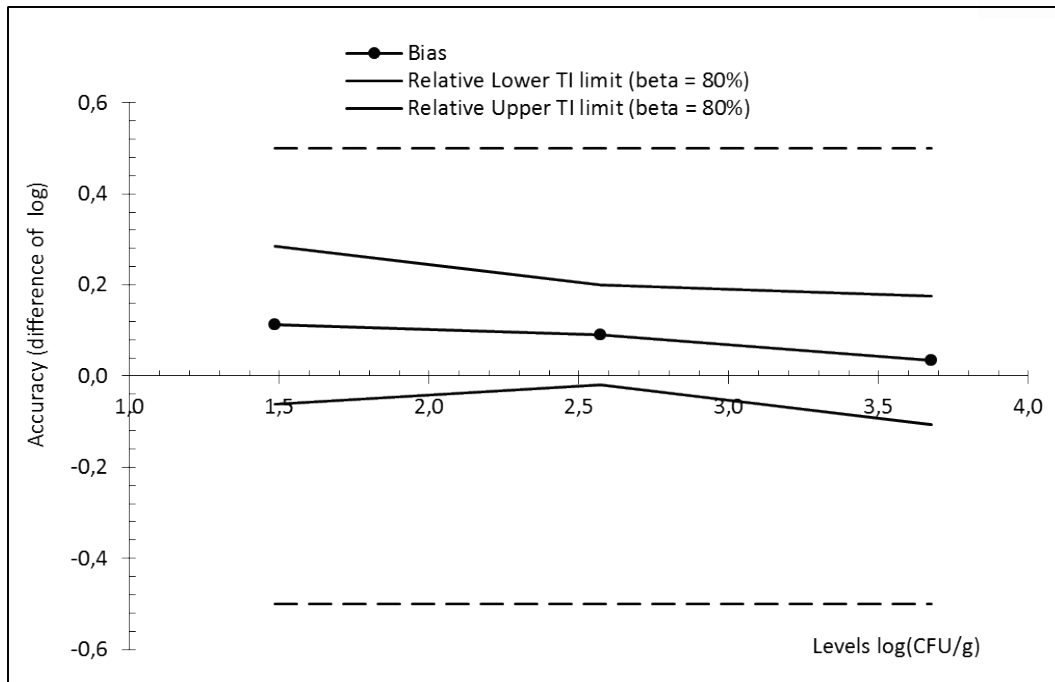


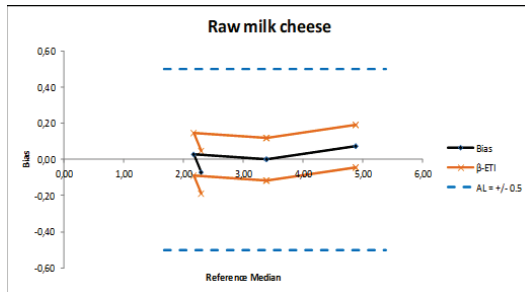
Figure 7. Graphical representation of the accuracy profile of the interlaboratory study for *E. coli* test at 44°, reference method was ISO 16649-2

CONCLUSION

According to the comparison and the interlaboratory study no statistical differences were found between RAPID'*E.coli* 2 Agar and the reference methods, ISO 4832:2006 and ISO 16649-2: 2001.

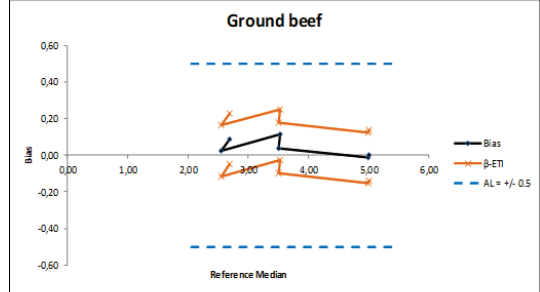
APPENDIX

Appendix 1. Accuracy profiles per category (reference coliform method at 30°C).



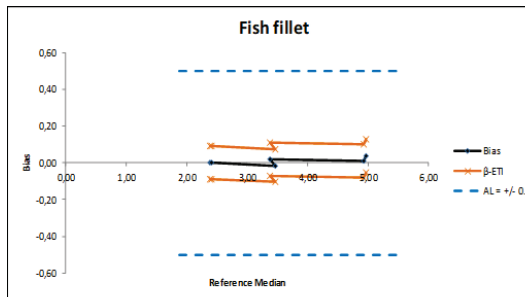
Sample Name	Reference Central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=±0.5 Acceptable	β -ETI compared to final AL Acceptable
2.30		-0.071	-0.188	0.046	YES	YES
2.18		0.028	-0.089	0.145	YES	YES
3.38		-0.090	-0.117	0.117	YES	YES
3.40		0.000	-0.117	0.117	YES	YES
4.89		0.073	-0.044	0.189	YES	YES
4.89		0.073	-0.044	0.189	YES	YES

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.123	0.081	YES	+/- 0.500



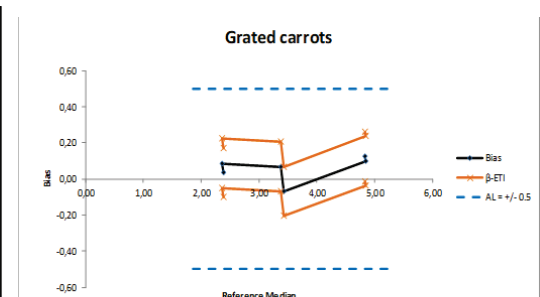
Sample Name	Reference Central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=±0.5 Acceptable	β -ETI compared to final AL Acceptable
2.69		0.088	-0.051	0.227	YES	YES
2.56		0.023	-0.115	0.162	YES	YES
3.53		0.112	-0.027	0.251	YES	YES
3.51		0.039	-0.100	0.178	YES	YES
4.99		-0.014	-0.152	0.125	YES	YES
5.00		0.000	-0.139	0.139	YES	YES

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.060	0.036	YES	+/- 0.500



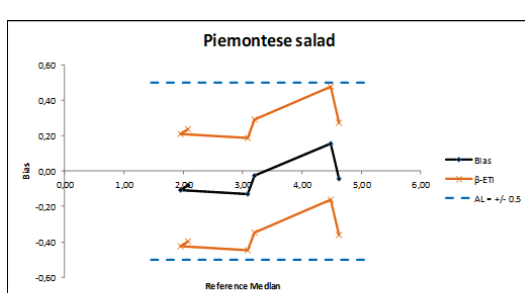
Sample Name	Reference Central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=±0.5 Acceptable	β -ETI compared to final AL Acceptable
2.38		0.000	-0.090	0.090	YES	YES
2.41		0.000	-0.090	0.090	YES	YES
3.46		-0.015	-0.105	0.074	YES	YES
3.38		0.018	-0.072	0.107	YES	YES
4.93		0.010	-0.079	0.100	YES	YES
4.96		0.036	-0.053	0.126	YES	YES

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.078	0.062	YES	+/- 0.500



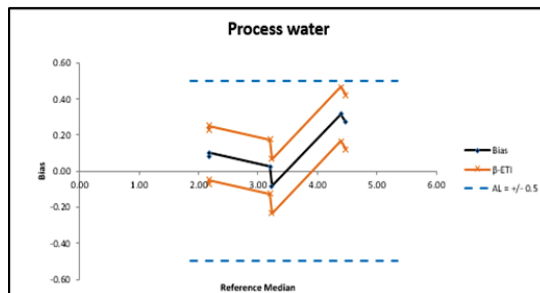
Sample Name	Reference Central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=±0.5 Acceptable	β -ETI compared to final AL Acceptable
2.38		0.035	-0.102	0.172	YES	YES
2.36		0.085	-0.052	0.223	YES	YES
3.38		0.067	-0.070	0.204	YES	YES
3.43		-0.070	-0.207	0.068	YES	YES
4.85		0.099	-0.038	0.237	YES	YES
4.83		0.123	-0.014	0.260	YES	YES

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.059	0.035	YES	+/- 0.500



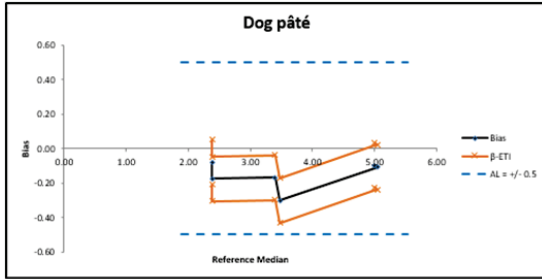
Sample Name	Reference Central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=±0.5 Acceptable	β -ETI compared to final AL Acceptable
2.08		-0.079	-0.387	0.238	YES	YES
1.95		-0.109	-0.427	0.208	YES	YES
3.08		-0.130	-0.447	0.188	YES	YES
3.20		-0.028	-0.346	0.280	YES	YES
4.48		0.156	-0.161	0.474	YES	YES
4.62		-0.043	-0.361	0.274	YES	YES

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.215	0.220	NO	+/- 0.500



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
2.18		0.079	-0.071	0.229	YES	YES
2.18		0.103	-0.047	0.253	YES	YES
3.20		0.026	-0.124	0.176	YES	YES
3.23		-0.084	-0.234	0.066	YES	YES
4.40		0.318	0.168	0.468	YES	YES
4.48		0.271	0.121	0.421	YES	YES

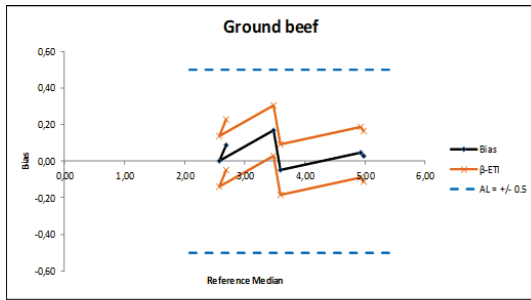
	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.072	0.104	YES	+/- 0.500



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
2.38		-0.079	-0.209	0.051	YES	YES
2.39		-0.176	-0.306	-0.046	YES	YES
3.40		-0.167	-0.237	-0.038	YES	YES
3.48		-0.301	-0.431	-0.171	YES	YES
5.04		-0.187	-0.237	0.023	YES	YES
5.00		-0.097	-0.227	0.033	YES	YES

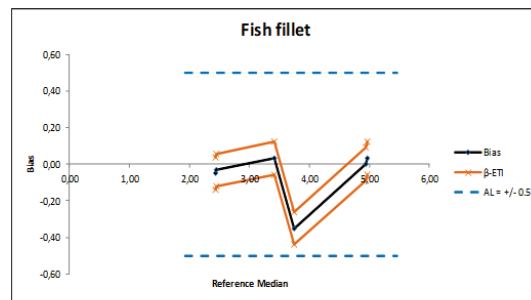
	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.103	0.090	YES	± 0.500

Appendix 2. Accuracy profiles per category (reference coliform method at 37°C).



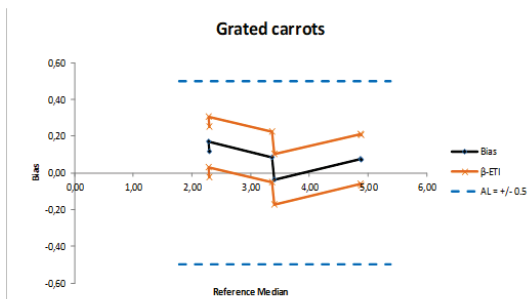
Sample Name	Reference central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=±0.5 Acceptable	β -ETI compared to final AL Acceptable
	2.69	0.088	-0.051	0.227	YES	YES
	2.58	0.000	-0.139	0.139	YES	YES
	3.48	0.186	0.028	0.305	YES	YES
	3.59	-0.047	-0.186	0.092	YES	YES
	4.93	0.048	-0.090	0.187	YES	YES
	4.97	0.027	-0.112	0.185	YES	YES

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.074	0.096	YES	+/- 0.500



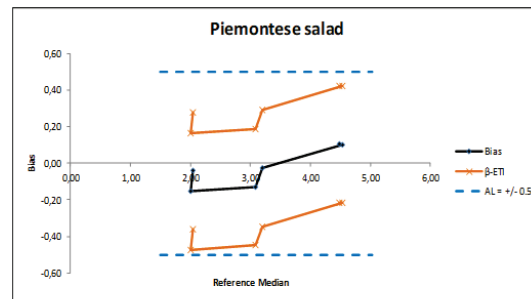
Sample Name	Reference Central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=±0.5 Acceptable	β -ETI compared to final AL Acceptable
	2.43	-0.051	-0.141	0.038	YES	YES
	2.45	-0.032	-0.122	0.057	YES	YES
	3.41	0.032	-0.057	0.122	YES	YES
	3.75	-0.350	-0.440	-0.261	YES	YES
	4.94	0.000	-0.090	0.090	YES	YES
	4.97	0.032	-0.068	0.121	YES	YES

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.099	0.062	YES	+/- 0.500



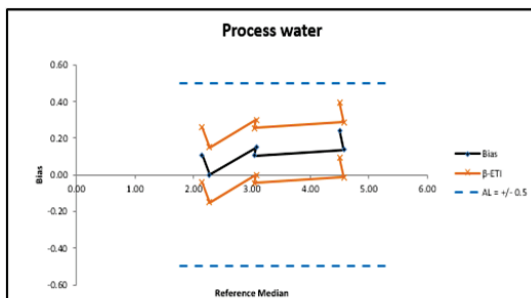
Sample Name	Reference Central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=±0.5 Acceptable	β -ETI compared to final AL Acceptable
	2.30	0.114	-0.023	0.251	YES	YES
	2.28	0.188	0.031	0.306	YES	YES
	3.36	0.005	-0.052	0.223	YES	YES
	3.40	-0.036	-0.173	0.101	YES	YES
	4.87	0.075	-0.062	0.212	YES	YES
	4.88	0.069	-0.069	0.206	YES	YES

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.068	0.095	YES	+/- 0.500



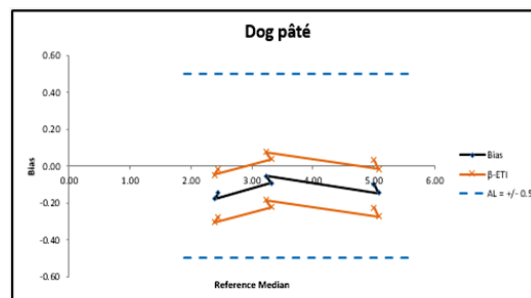
Sample Name	Reference Central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=±0.5 Acceptable	β -ETI compared to final AL Acceptable
	2.04	-0.041	-0.359	0.276	YES	YES
	2.00	-0.155	-0.472	0.163	YES	YES
	3.08	-0.130	-0.447	0.188	YES	YES
	3.20	-0.028	-0.346	0.290	YES	YES
	4.53	0.102	-0.216	0.420	YES	YES
	4.48	0.103	-0.215	0.420	YES	YES

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.184	0.220	NO	+/- 0.500



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
	2.15	0.109	-0.041	0.259	YES	YES
	2.28	0.000	-0.150	0.150	YES	YES
	3.08	0.151	0.001	0.301	YES	YES
	3.04	0.105	-0.045	0.255	YES	YES
	4.58	0.138	-0.014	0.286	YES	YES
	4.51	0.243	0.093	0.393	YES	YES

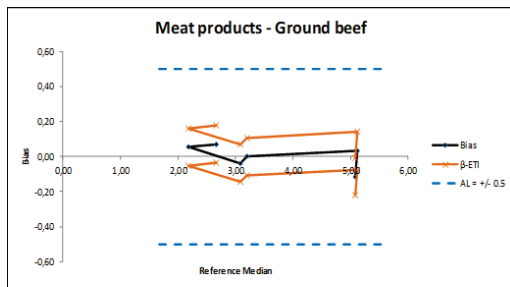
	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.090	0.104	YES	+/- 0.500



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
	2.45	-0.146	-0.276	-0.016	YES	YES
	2.38	-0.178	-0.308	-0.046	YES	YES
	3.32	-0.092	-0.222	0.039	YES	YES
	3.23	-0.054	-0.184	0.076	YES	YES
	5.08	-0.145	-0.275	-0.015	YES	YES
	5.00	-0.097	-0.227	0.033	YES	YES

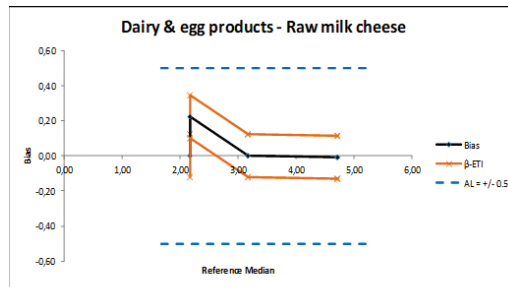
	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.104	0.090	YES	+/- 0.500

Appendix 3. Accuracy profiles per category (reference *E. coli* method at 37°C).



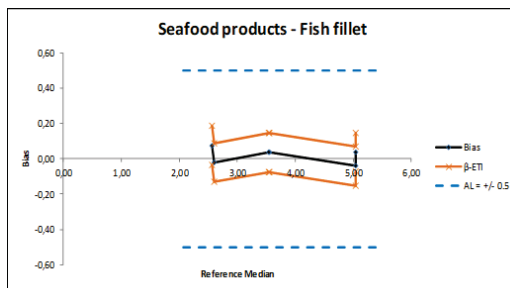
Sample Name	Reference Central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=0.5 Acceptable	β -ETI compared to final AL Acceptable
2.66	0.070	-0.037	0.176	YES	YES	
2.18	0.054	-0.052	0.161	YES	YES	
3.08	-0.038	-0.145	0.069	YES	YES	
3.20	0.000	-0.107	0.107	YES	YES	
5.11	0.032	-0.075	0.139	YES	YES	
5.08	-0.115	-0.222	-0.009	YES	YES	

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.085	0.074	YES	+/- 0.500



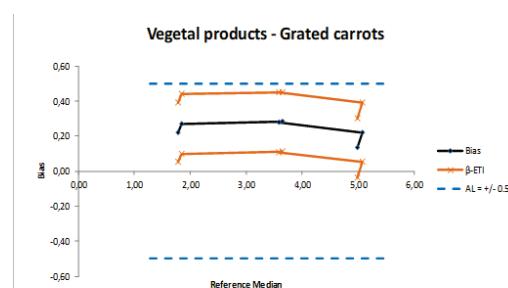
Sample Name	Reference Central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=0.5 Acceptable	β -ETI compared to final AL Acceptable
2.18	0.000	-0.121	0.121	YES	YES	
2.18	0.222	0.101	0.343	YES	YES	
3.18	0.000	-0.121	0.121	YES	YES	
3.18	0.000	-0.121	0.121	YES	YES	
4.71	-0.009	-0.130	0.113	YES	YES	
4.72	-0.008	-0.130	0.113	YES	YES	

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.057	0.064	YES	+/- 0.500



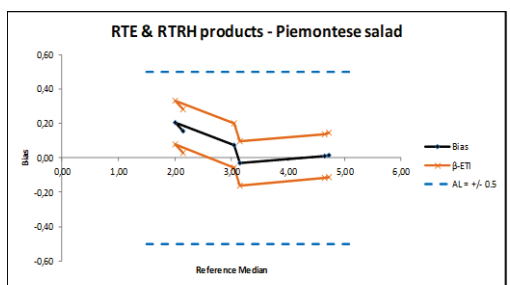
Sample Name	Reference Central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=0.5 Acceptable	β -ETI compared to final AL Acceptable
2.57	0.075	-0.034	0.185	YES	YES	
2.60	-0.022	-0.132	0.087	YES	YES	
3.56	0.035	-0.075	0.144	YES	YES	
3.54	0.036	-0.074	0.145	YES	YES	
5.04	-0.041	-0.151	0.068	YES	YES	
5.04	0.038	-0.072	0.148	YES	YES	

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.072	0.076	YES	+/- 0.500



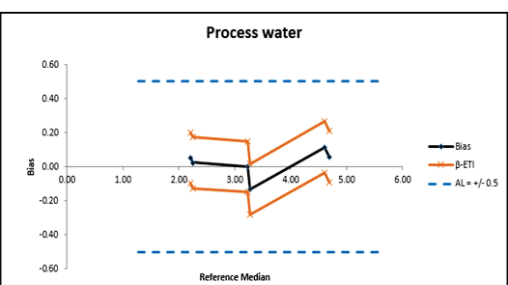
Sample Name	Reference Central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=0.5 Acceptable	β -ETI compared to final AL Acceptable
1.78	0.222	0.052	0.382	YES	YES	
1.85	0.269	0.098	0.439	YES	YES	
3.64	0.281	0.110	0.451	YES	YES	
3.58	0.278	0.107	0.448	YES	YES	
5.08	0.222	0.052	0.382	YES	YES	
4.98	0.132	-0.039	0.302	YES	YES	

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.100	0.118	YES	+/- 0.500



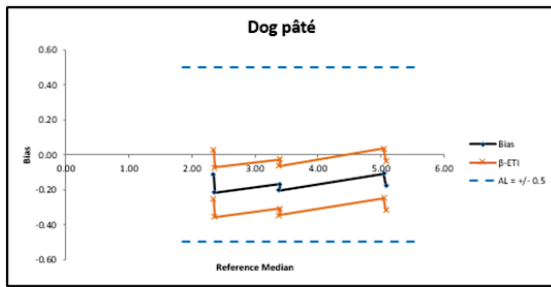
Sample Name	Reference Central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=0.5 Acceptable	β -ETI compared to final AL Acceptable
2.15	0.155	0.026	0.283	YES	YES	
2.00	0.204	0.076	0.333	YES	YES	
3.04	0.073	-0.056	0.201	YES	YES	
3.15	-0.032	-0.161	0.086	YES	YES	
4.65	0.010	-0.119	0.138	YES	YES	
4.72	0.016	-0.112	0.145	YES	YES	

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.080	0.089	YES	+/- 0.500



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
2.20	0.051	-0.099	0.201	YES	YES	
2.26	0.023	-0.127	0.174	YES	YES	
3.23	0.000	-0.150	0.150	YES	YES	
3.28	-0.133	-0.283	0.018	YES	YES	
4.60	0.114	-0.035	0.254	YES	YES	
4.69	0.058	-0.092	0.208	YES	YES	

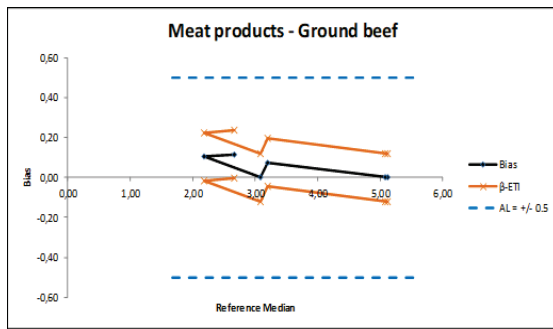
	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.085	0.104	YES	+/- 0.500



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
	2.34	-0.112	-0.253	0.030	YES	YES
	2.36	-0.216	-0.357	-0.074	YES	YES
	3.40	-0.167	-0.309	-0.026	YES	YES
	3.38	-0.204	-0.346	-0.063	YES	YES
	5.04	-0.107	-0.248	0.035	YES	YES
	5.08	-0.176	-0.318	-0.035	YES	YES

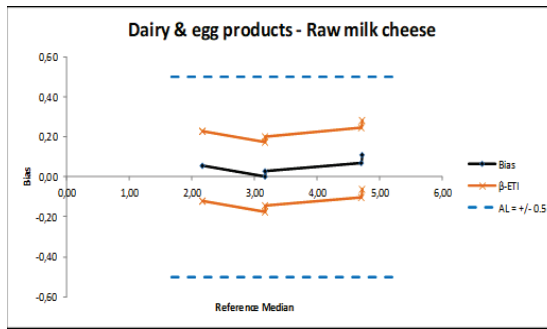
	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.093	0.038	YES	± 0.500

Appendix 4. Accuracy profiles per category (reference *E. coli* method at 44°C).



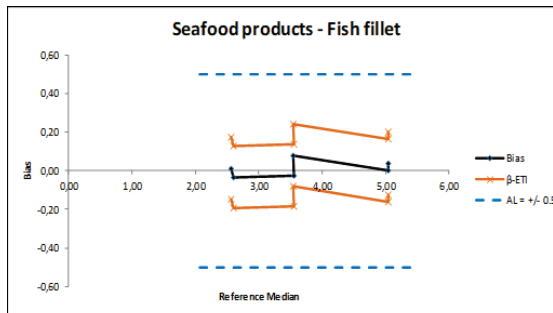
Sample Name	Reference central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=±0.5 Acceptable	β -ETI compared to final AL Acceptable
	2,66	0,115	-0,004	0,235	YES	YES
	2,18	0,103	-0,017	0,222	YES	YES
	3,08	0,000	-0,120	0,120	YES	YES
	3,20	0,075	-0,045	0,194	YES	YES
	5,11	0,000	-0,120	0,120	YES	YES
	5,08	0,000	-0,120	0,120	YES	YES

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0,085	0,083	YES	+/- 0,500



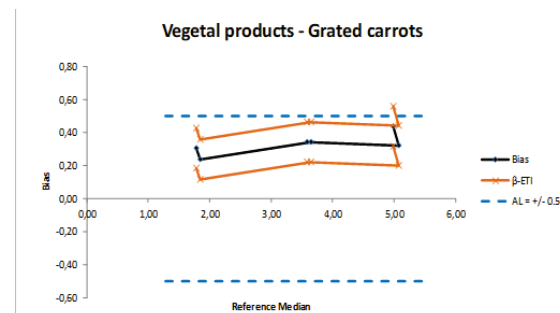
Sample Name	Reference Central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=±0.5 Acceptable	β -ETI compared to final AL Acceptable
	2,18	0,054	-0,119	0,228	YES	YES
	2,18	0,054	-0,119	0,228	YES	YES
	3,18	0,000	-0,173	0,173	YES	YES
	3,18	0,028	-0,145	0,201	YES	YES
	4,71	0,071	-0,103	0,244	YES	YES
	4,72	0,110	-0,083	0,283	YES	YES

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0,057	0,120	YES	+/- 0,500



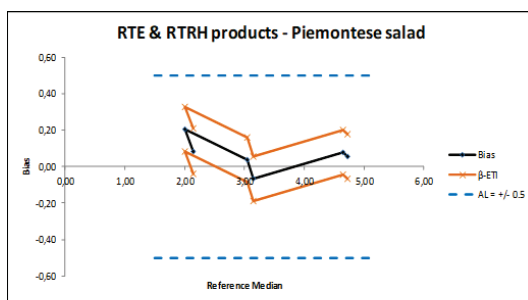
Sample Name	Reference Central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=±0.5 Acceptable	β -ETI compared to final AL Acceptable
	2,57	0,012	-0,150	0,173	YES	YES
	2,60	-0,034	-0,196	0,128	YES	YES
	3,56	-0,025	-0,187	0,137	YES	YES
	3,54	0,079	-0,083	0,241	YES	YES
	5,04	0,000	-0,162	0,162	YES	YES
	5,04	0,038	-0,124	0,199	YES	YES

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0,072	0,112	YES	+/- 0,500



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
	1,78	0,301	0,190	0,422	YES	YES
	1,85	0,234	0,113	0,355	YES	YES
	3,64	0,339	0,218	0,460	YES	YES
	3,64	0,339	0,218	0,461	YES	YES
	5,08	0,319	0,197	0,440	YES	YES
	4,98	0,433	0,311	0,554	NO	NO

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0,100	0,084	YES	+/- 0,500



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
	2,15	0,084	-0,038	0,207	YES	YES
	2,00	0,204	0,081	0,327	YES	YES
	3,04	0,038	-0,085	0,160	YES	YES
	3,15	-0,067	-0,190	0,056	YES	YES
	4,65	0,079	-0,044	0,202	YES	YES
	4,72	0,054	-0,069	0,177	YES	YES

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0,080	0,085	YES	+/- 0,500