NordVal International / NMKL
c/o Danish Technical University
Kemitorvet B201
DK-2800 Kgs Lyngby, Denmark
www.nmkl.org

NordVal International Certificate

Issued for:	RAPID'*Staph*
NordVal No:	049
First approval date:	20 May 2018
Valid until:	20 May 2020

RAPID'*Staph*

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 method was ISO 6888-1:1999 Horizontal method for the enumeration of coagulase-positive Staphylococci (Staphylococcus aureus and other species). Part 1: Technique using Baird-Parker Agar medium.

NordVal International has studied the enclosures to the application and evaluated the results obtained in the validations conducted by ADRIA Développement, France 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 when prolonging the incubation time with additional 24h when doubtful colonies are obtained after the first 24h. The results document no statistical difference in the performances between the RAPID'*Staph* and the reference method.

Date: 20 May 2018

Yours sincerely,

Hilde Skår Norli
Chair of NordVal International

Nina Skall Nielsen
NMKL Secretary General

PRINCIPLE OF THE METHOD:

RAPID'*Staph* is based on enumeration on an optimised Baird-Parker formula for enumeration of S. aureus in 24 h at 37°C. Some Staphylococcus coagulase positive strains can give colonies with a small or without halo after 24 hours of incubation.
An additional incubation of 24 hours could be necessary to see the halo.

Using RAPID'*Staph* the typical staphylococci produce gray to black colonies that are shiny and cover (1-2 mm) with a clear 2 - 5 mm halo due to proteolysis of the egg yolk. Presumptive colonies can be confirmed using one of the following methods:

- PastorexTM Staph-Plus Latex Agglutination Test;
- Spot inoculation of Baird-Parker + RPF agar to confirm until 12 colonies on the same plate.

FIELD OF APPLICATION:

The method is applicable for enumeration of coagulase-positive Staphylococci (Staphylococcus aureus and other species) in a broad range of foods.

HISTORY:

The RAPID'Staph method was validated first time in February 2005 for a broad range of foods and has been certified by other organisations since then.

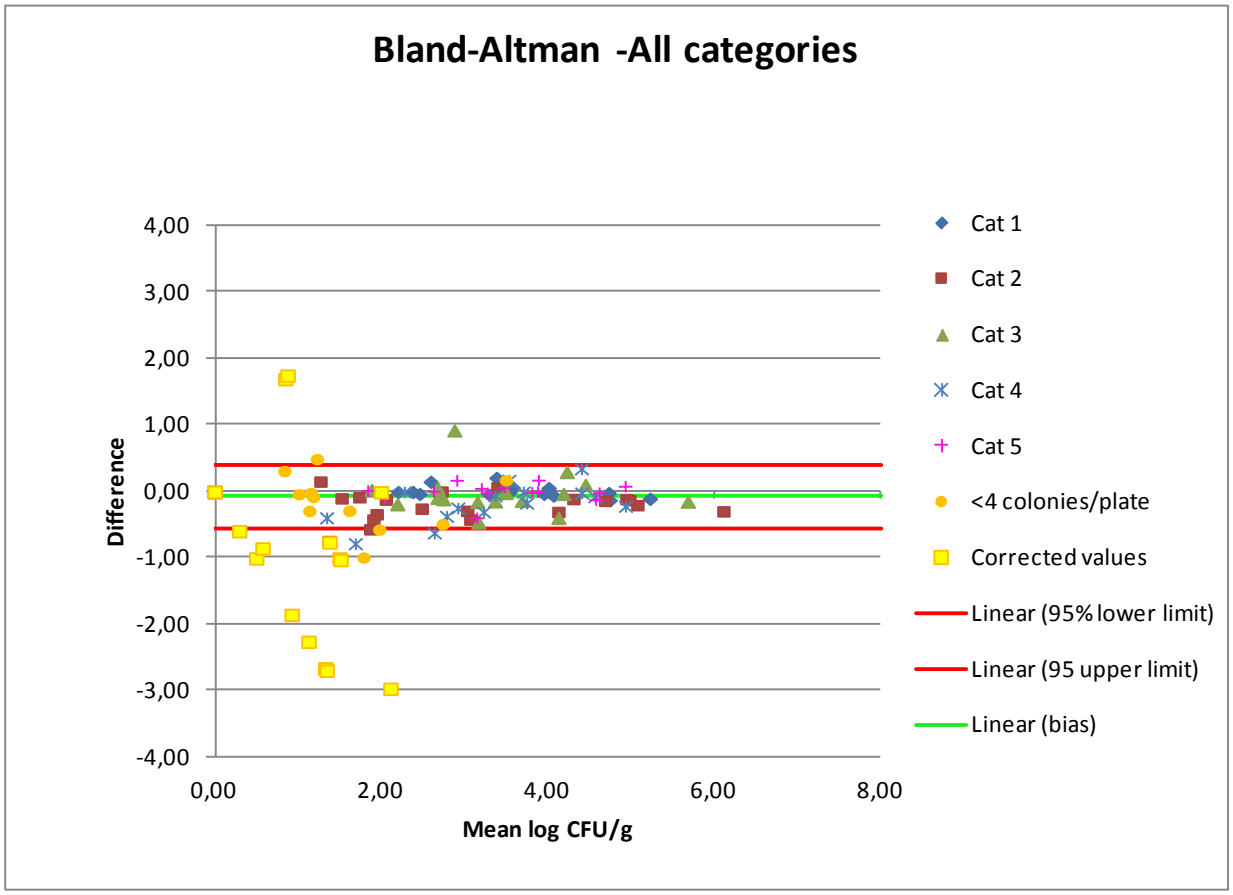
In 2018, RAPID'Staph has been revalidated in order to comply with EN ISO 16140-2:2016. This is the first time RAPID'Staph is certified by NordVal International.

METHOD COMPARISON STUDY

Relative trueness study

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 total *Staphylococcus* in foods by the alternative and the reference method, respectively. A total of 81 interpretable results from 5 food categories including composite foods (ready to eat, ready to reheat), meat products, dairy products, egg products and seafood products were obtained. The samples were both naturally and artificially contaminated.

Figure 1: Bland-Altman difference plot for all categories. Comparison of reference method versus Alternative method



Conclusions of the relative trueness study:

The results of the Bland-Altman Plot (Figure 1) provides a visual observation on the amount of bias and extreme results. It is expected that no more than one in 20 (5%) data values will lie outside the upper and lower limits. In this study, more than 5% of the results fall below the lower limit. For 9 samples, non-typical colonies were present on BP Agar plates, and confirmed positive as coagulase positive *Staphylococci* using the coagulase test in rabbit plasma. For these the incubation time was 24, not 48h.

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. This study is conducted using artificially contaminated samples. One type per category is tested for this.

Food matrices

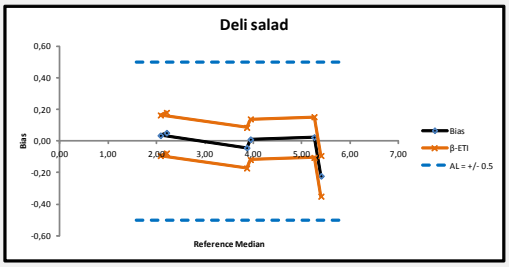
For each of the 5 food categories~~,~~ one type of food/sample was selected and two batches of each type were inoculated. Of 6 samples, 2 were inoculated at a low level (100 cfu/g), 2 at a medium level (5000 cfu/g) and 2 at a high level (10^5 cfu/g) of contamination. For each of the 6 samples per category and two batches of each, 5 replicate test portions were tested.

The observed profiles are within the Acceptance Limit, AL of ± 0.5 log cfu/g.

All the accuracy profiles, shown in figure 2, fulfil the performance criteria and the alternative method is accepted as being equivalent to the reference method.

Figure 2: Accuracy profiles (β = 80%, AL = 0.5)

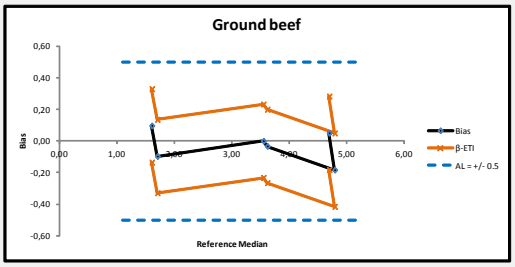
(Food) Category	RTE
(Food) Type	Deli salad



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
2419-2423	2,20	0,051	-0,077	0,180	YES	YES
2434-2438	2,08	0,035	-0,094	0,163	YES	YES
2424-2428	3,86	-0,044	-0,172	0,085	YES	YES
2439-2443	3,94	0,010	-0,119	0,138	YES	YES
2429-2433	5,26	0,023	-0,105	0,152	YES	YES
2444-2448	5,40	-0,222	-0,350	-0,093	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0,102	0,089	YES	+/- 0,500

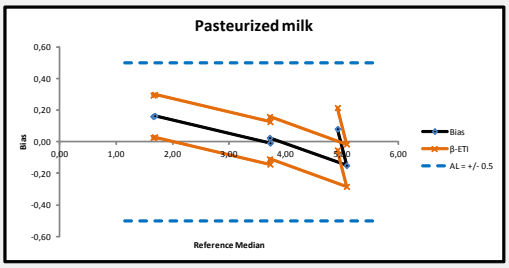
(Food) Category	Meat products
(Food) Type	Ground beef



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
4582-4586	1,60	0,097	-0,136	0,329	YES	YES
4597-4601	1,70	-0,097	-0,329	0,136	YES	YES
4587-4591	3,54	0,000	-0,232	0,232	YES	YES
4602-4606	3,61	-0,033	-0,265	0,199	YES	YES
4592-4596	4,79	-0,183	-0,416	0,049	YES	YES
4607-4611	4,69	0,050	-0,182	0,283	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0,114	0,161	YES	+/- 0,500

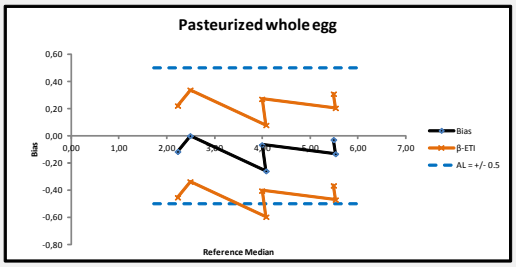
(Food) Category	Dairy product
(Food) Type	Pasteurized milk



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
4884-4888	1,65	0,160	0,025	0,294	YES	YES
4899-4903	1,68	0,164	0,030	0,298	YES	YES
4889-4893	3,72	-0,008	-0,143	0,126	YES	YES
4904-4908	3,72	0,024	-0,110	0,159	YES	YES
4894-4898	5,08	-0,150	-0,284	-0,016	YES	YES
4909-4913	4,92	0,081	-0,053	0,215	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0,110	0,093	YES	+/- 0,500

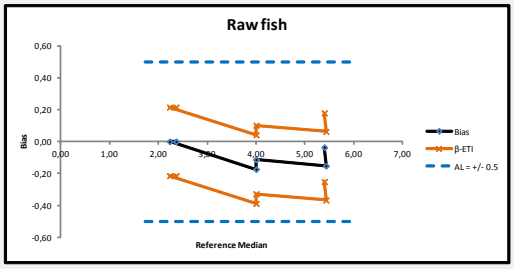
(Food) Category	Egg products and miscellaneous
(Food) Type	Pasteurized whole egg



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
5611-5615	2,23	-0,117	-0,454	0,221	YES	YES
5596-5600	2,49	0,000	-0,338	0,338	YES	YES
5601-5605	4,08	-0,260	-0,597	0,078	NO	NO
5616-5620	3,99	-0,067	-0,405	0,271	YES	YES
5606-5610	5,53	-0,134	-0,471	0,204	YES	YES
	5,49	-0,029	-0,367	0,309	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0,098	0,234	YES	+/- 0,500

(Food) Category	Fishery products
(Food) Type	Raw 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
5869-5873	2,36	0,000	-0,215	0,215	YES	YES
5884-5888	2,23	0,000	-0,215	0,215	YES	YES
5874-5878	4,00	-0,174	-0,389	0,041	YES	YES
5889-5893	4,00	-0,114	-0,329	0,102	YES	YES
5879-5883	5,43	-0,153	-0,368	0,062	YES	YES
5894-5898	5,40	-0,036	-0,251	0,179	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0,112	0,149	YES	+/- 0,500

Selectivity; Inclusivity and exclusivity

Inclusivity: 50 strains of *Staphylococcus* have been tested in two studies. 11 strains gave non characteristic colonies on RAPID'Staph Agar (without halo) and on BP agar. For 5 of them, characteristic colonies (with halo) were obtained after 48 h incubation using BP Agar (ISO 6888-1 method) and RAPID'Staph Agar. After 48h, 6 strains gave still non-characteristic colonies, but were confirmed as coagulase positive *Staphylococci* (using Rabbit plasma).

The inclusivity study shows that some Staphylococcus coagulase positive can give non-characteristic colonies after 24 hours, thus a prolongation up to 48 hours incubation time may be necessary to see the halo. If there is any doubt on the colony aspect, it is recommended to perform a confirmation testing on the suspect colonies.

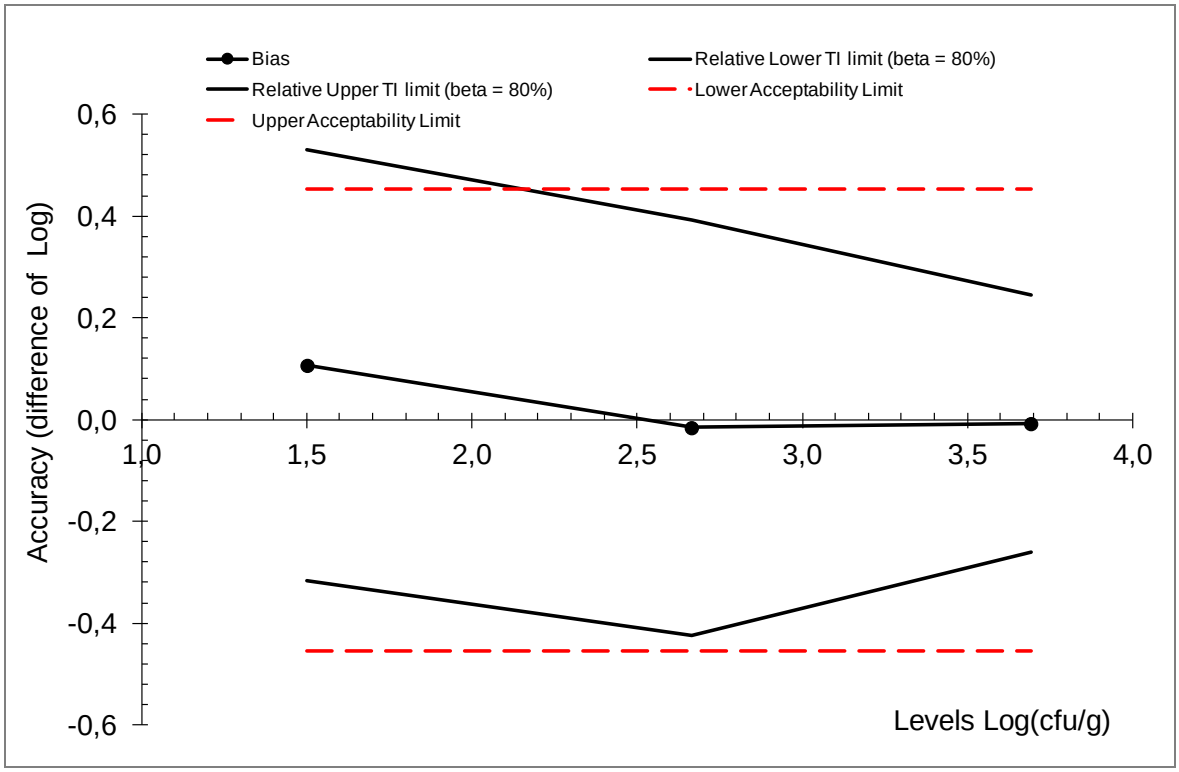
Exclusivity: 30 strains of other genera were tested. There was no typical reaction of Staphylococcus for these strains.

COLLABORATIVE STUDY

Twelve collaborative laboratories participated in the collaborative study from 2004. *Staphylococcus aureus* 501 isolated from raw milk used for inoculation of pasteurized skimmed milk. Eight samples were prepared, two at each of four different levels: zero, lower (log 1-2 CFU/ml), middle (log 2-3 CFU/ml) and higher (log 3-4 CFU/ml). The results are given in the table below.

Levels	No. of labs	Mean (log cfu/g)	S_R (log cfu/g)	Bias (log cfu/g)	Lower limit (log cfu/g)	Upper limit (log cfu/g)	± AL (log cfu/g)
1.50	11	1.61	0.28	0.11	-0.32	0.53	0.5
2.66	12	2.65	0.30	-0.02	-0.43	0.39	0.5
3.69	12	3.68	0.11	-0.01	-0.26	0.25	0.5

Figure 3. Graphical representation of the accuracy profile of the interlaboratory study for *Staphylococcus*



The results of the interlaboratory show that the method performs equivalent to the reference method for levels above 2.0 log cfu/g.

CONCLUSION:

According to the comparison and the interlaboratory study the RAPID'*Staph* provide equivalent results to the reference method ISO 6888-1 (1999) for levels of 2.0 log cfu/g and above. However, in some cases prolongation up to 48 hours incubation time may be necessary to see the halo characteristic for *Staphylococcus.*