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NordVal International Certificate

Issued for:	TRANSIA® PLATE *Salmonella* Gold
NordVal No:	001
First approval date:	14 June 2001
Renewal date:	5 October 2017
Valid until:	5 October 2019

TRANSIA® PLATE *Salmonella* Gold

Manufactured and Supplied by:
BioControl
12822 SE 32nd Street
Bellevue
WA 98005
USA.

fulfils the requirements of the NordVal validation protocol. The performance of the TRANSIA® PLATE *Salmonella* Gold has been compared with the following reference method:

- EN ISO 6579:2002: Microbiology of food and feeding stuffs. Horizontal method for the detection of *Salmonella* spp

The results document no statistical difference in the performances between the methods.

Date: 4 October 2017

Yours sincerely,

Hilde Skår Norli
Chair of NordVal International

Nina Skall Nielsen
NMKL Secretary General

PRINCIPLE OF THE METHOD

TRANSIA® PLATE *Salmonella* Gold is based on a on a three-step, sandwich-type ELISA (Enzyme Linked ImmunoSorbent Assay) using:

- a microtitre plate with divisible strips coated with antibodies specific for *Salmonella*
- and ready-to use reagents.

The method describes:

- Enrichment on buffered peptone water (BPW) incubated for 16-20 h at 37°C ± 1°C
- Inoculation of 0.1 mL of the pre-culture broth in 10 mL of Rappaport Vassiliadis Soya (RVS) broth with or without Novobiocin solution. For matrices with known high levels of competitive bacteria, such as *E. coli,* addition of Novobiocin solution might reduce the possibility of competitive overload. As an optional procedure: Add 25 µL of 0.45% Novobiocin solution in 10 mL RVS. Inoculate 0.1 mL of the pre-culture broth in RVS+N tubes .
- Incubate at 41.5°C ± 1°C for 18-24 h, followed by
- TRANSIA® PLATE *Salmonella* Gold test after heating of 1 to 2 mL of the enrichment broth RVS in boiling water for 20 minutes.
- The reading of the microtitre plate is carried out using a spectrophotometer at a wavelength of 450 nm.

FIELD OF APPLICATION

The method has been tested on foods, feeds and environmental samples.

VALIDATION HISTORY

The TRANSIA® PLATE *Salmonella* Gold was first approved in 2001 and reviewed based on additional studies in 2005 and 2009. In 2015, the certificate was renewed with a technical modification which was validated in 2016 and included in the certificate of 2017. The validations are carried out according to EN ISO 16140 and ISO 16140-2:2016.

In 2009, the TRANSIA® PLATE *Salmonella* Gold method was modified at the immuno-enzymatic step: the substrate and the chromogen were combined into a single reagent and a step of extraction was added to the protocol (introduction of the non selective additive to the sample preparation before heating). Additional tests have been conducted ensuring that the modifications made have not diminished the performance of the method.

In 2015, Novobiocin has been included as an optional addition to the Rappaport Vassiliadis Soya (RVS). The optional change was made to improve the selectivity of the method for samples that are highly contaminated with competitive microflora. The inclusivity study of 107 target strains and the exclusivity study of 43 non-target strains showed satisfactory results. The studies for the extension and the sensitivity study were carried out in 2016 according to the requirements of the ISO 16140-2:2016.

The results of the different studies are included in this certificate.

COMPARISON STUDY

All the studies have been conducted by ADRIA Dévelopment, Cedex, France.

Accuracy, sensitivity, specificity

From the study conducted in 2004: 429 samples were analysed of which 69 were naturally contaminated, 118 artificially contaminated, and 242 non-contaminated, representing the following categories: meat products, dairy products, seafood products, vegetables and environmental samples.

The following results were obtained:

Table 1: Results after screening

Matrix	PA	NA	ND	PD	Sum	Relative AC %	Relative SE %	Relative SP %	Kappa
Meat	31	78	0	0	**109**	100	100	100	1.0
Dairy Products	35	30	0	0	**65**	100	100	100	1.0
Seafood & vegetables	30	34	0	0	**64**	100	100	100	1.0
Eggproducts and pastries	30	32	0	0	**62**	100	100	100	1.0
Feed	30	32	1	0	**69**	98.6	96.7	100	1.0
Environment	31	29	0	0	**60**	100	100	100	1.0
Total	**186**	**242**	**1**	**0**	**429**	**99.8**	**99.5**	**100**	**1.0**

PA = number of obtained results that are positive with both the alternative and the reference method

NA = number of obtained results that are negative with both the alternative and the reference method.

ND = number of obtained results that are negative with the alternative method and positive with the reference method (possible false negative)

PD = number of obtained results that are positive with the alternative method and negative with the reference method (possible false positive)

Relative AC = The relative accuracy; the degree of correspondence between the response obtained by the alternative method and the reference method.

Relative SE = The relative sensitivity; the ability of the alternative method to detect the analyte compared to the reference method

Relative SP = The relative specificity is the ability of the alternative method not to detect the target microorganism when it is not detected by the reference method.

Kappa = The degree of agreement between the alternative method and the reference method, kappa of 0.80 or higher is considered to be very good agreement.

After the confirmation, the feed sample which was obtained as negative after the screening was negative after the confirmation when presumed positive.

From the study in 2009: Due to method modifications a complementary study was carried out in 2009. The following results were obtained.

Table 2: Results after the screening

Matrix	PA	NA	ND	PD	Sum	Relative AC %	Relative SE %	Relative SP %	Kappa
Meat	11	11	0	0	**22**	100	100	100	1.0
Dairy Products	11	16	0	0	**27**	100	100	100	1.0
Seafood & vegetables	11	16	0	0	**27**	100	100	100	1.0
Eggproducts and pastries	10	18	0	0	**28**	100	100	100	1.0
Feed	10	10	0	0	**20**	100	100	100	1.0
Environment	10	14	0	0	**24**	100	100	100	1.0
Total	**63**	**85**	**0**	**0**	**148**	**100**	**100**	**100**	**1.0**

The results obtained by the two methods were in full agreement.

From the study in 2016:

Table 3 – Results after screening and confirmation; relative accuracy (AC), relative sensitivity (SE) and relative specificity (SP) and kappa (agreement between methods)

Category	Type	PA	NA	PD	ND	AC %	SE %	SP %	Kappa*
Ready-to-eat and Ready-to reheat	Ready-to-eat	11	9	0	1	95.2	91.7	100.0	0.91
	Ready-to-reheat	9	11	0	1	95.2	90.0	100.0	0.91
	Marinated and smoked products	8	12	0	0	100.0	100.0	100.0	1.00
	Total	28	32	0	2	96.8	93.3	100.0	0.91
Meat Products	Meat products	13	14	0	0	100.0	100.0	100.0	1.00
	Poultry products	8	12	0	0	100.0	100.0	100.0	1.00
	Delicatessen	9	15	0	0	100.0	100.0	100.0	1.00
	Total	30	41	0	0	100.0	100.0	100.0	1.00
Dairy products	Pasteurised dairy products	9	11	0	0	100.0	100.0	100.0	1.00
	Raw dairy products	10	11	0	0	100.0	100.0	100.0	1.00
	Milk powders and dairy based products	11	17	0	0	100.0	100.0	100.0	1.00
	Total	30	39	0	0	100.0	100.0	100.0	1.00
Vegetables and seafood products	Raw fishery products	11	9	0	0	100.0	100.0	100.0	1.00
	Fresh cut vegetables	6	13	0	1	95.0	85.7	100.0	0.91
	Raw vegetable products	12	8	0	0	100.0	100.0	100.0	1.00
	Total	29	30	0	1	98.3	96.7	100.0	0.97
Ingredients and specific products	Raw materials	9	15	0	0	100.0	100.0	100.0	1.00
	Infant formula and infant cereal	9	11	0	0	100.0	100.0	100.0	1.00
	Pasteurised egg products and egg powders	12	10	0	1	95.7	92.3	100.0	0.91
	Total	30	36	0	1	98.5	96.8	100.0	0.97
Feed products	Products for pet	10	10	0	0	100.0	100.0	100.0	1.00
	Products for cattle	10	15	0	1	96.2	90.9	100.0	0.93
	Raw material	10	12	0	0	100.0	100.0	100.0	1.00
	Total	30	37	0	1	98.5	96.8	100.0	0.97
Environ-mental samples	Process water	12	18	0	0	100.0	100.0	100.0	1.00
	Dusts	10	21	0	0	100.0	100.0	100.0	1.00
	Wipes	11	17	0	1	96.6	91.7	100.0	0.93
	Total	33	56	0	1	98.9	97.1	100.0	0.98
All Categories		210	271	0	6	98.8	97.2	100.0	0.98

*The negative deviations, ND, found negative with the alternative method and positive with the reference method were confirmed negative;

PA = number of obtained results that are positive with both the alternative and the reference method

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ND = number of obtained results that are negative with the alternative method and positive with the reference method (possible false negative)

PD = number of obtained results that are positive with the alternative method and negative with the reference method

(possible false positive)

Relative AC = The relative accuracy; the degree of correspondence between the response obtained by the alternative method and the reference method.

Relative SE = The relative sensitivity; the ability of the alternative method to detect the analyte compared to the reference method

Relative SP = The relative specificity is the ability of the alternative method not to detect the target microorganism when it is not detected by the reference method.

Kappa = The degree of agreement between the alternative method and the reference method, kappa of 0.80 or higher is considered to be very good agreement.

The statistical test, Cohen's kappa, κ, is used as an estimate for the agreement between the methods. For method validation, "very good agreement" is required, i.e. $\kappa > 0.80$. In this study, kappa was above 0.80 for all levels, indicating very good agreement between the reference method and the alternative method.

The Acceptability Limit (AL) in ISO 16140-2:2016 is not based on statistical analysis of the data, but on experience from many validations; tabled AL values depending on the number of categories.

Table 4: Analyses of discordant results according to the ISO 16140-2

Type	PD=FP	ND=FN	TP	FN-(FP+TP)	AL	(FN+TP)+FP	AL
Ready-to- eat and ready-to-reheat	0	2	0	2	3	2	6
Meat products	0	0	0	0	3	0	6
Dairy products	0	0	0	0	3	0	6
Vegetables and seafood products	0	1	0	1	3	1	6
Ingredients and specific products	0	1	0	1	3	1	6
Feed products	0	1	0	1	3	1	6
Environmental samples	0	1	0	1	3	1	6
Total	0	6	0	6	6	6	18

FP = false positive, presumptive false positive confirmed as false positive
TP = true positive, presumptive false positive confirmed as true positive
FN = false negative (the reference method is positive)

The observed values (FN- (FP + TP)) and ((FN + TP) + FP) are below the acceptability limit for each category and equal to the acceptability limit for all the categories.

Detection Level and RLOD

In 2004, different matrices were analysed 6 times at 4 different contamination levels by both methods. The detection level was found to be 1-10 cfu in a sample of 25 g or 25 ml for all matrices.
In 2016, an additional RLOD study was performed showing that the RLOD are below the AL fixed at 1.5 for paired studies for all the tested matrix / strain pairs.

Inclusivity /exclusivity

Inclusivity: In 2001, 70 strains of *Salmonella* were tested. Two strains of *Salmonella arizonae* serovar: IIIa 48:z4 z23, among 11 *Salmonella arizonae* and *Salmonella diarizonae* tested, were obtained negative. One strain of *Salmonella kedougou,* among fours trains tested, was obtained negative.
In 2005, 50 strains of *Salmonella* were detected out of 50 tested.
In 2009, 10 strains of *Salmonella* were tested. One of the strains was not detected by the alternative method (*Salmonella arizonae* serovar: IIIa 48:z4 z23). Another strain of *Salmonella arizonae* servar: IIIa 51:z4 z23 were obtained positive.
In 2015/16: 105 strains of *Salmonella* were detected out of 105 tested.

Exclusivity: The study of the 47 non-*Salmonella* strains in 2001, the 30 non-*Salmonella* strains in 2005 and the 37 non-*Salmonella* strains in 2015/16 by the TRANSIA® PLATE *Salmonella* Gold did not detect the presence of any cross-reaction.

Conclusion method comparison study

The method comparison study corresponds to a paired study design as the alternative and reference methods do have the same primary enrichment procedure.

The relative sensitivity, relative specificity, relative accuracy and agreement between the alternative method and the reference method are satisfactory and within the acceptability limit according to ISO 16140-2:2016. Further, the selectivity (inclusivity and exclusivity) for the more than 100 target strains and the 50 non-target strains tested were satisfactory.

The Relative Levels of Detection (RLOD) are all below the AL fixed at 1.5 for the paired data study whatever the matrix/strain pairs and the protocol.

It is possible to store the RVS broth for 48 h at 2 - 8°C for all the categories and the RVS+N broth for 48 h at 2-8°C for all categories, except for ready to eat and ready to reheat products.

The TRANSIA® Plate *Salmonella* GOLD allows a two-day screening of the negative samples.

INTERLABORATORY STUDY:

The interlaboratory study was conducted in 2004.
Number of laboratories: 11, however one laboratory did not receive the samples in time and did not carry out the analysis.

The analyses were performed on samples of pasteurized milk, artificially contaminated with a strain of *Salmonella Typhimurium* at the following levels:

- 0 cfu/25 ml
- 1-10 cfu/25 ml
- 10-50 cfu/25 ml

The laboratories analysed 8 replicates for each level using both the alternative method and the reference method. The following results were obtained – Table 5 and 6:

Table 5 Summary of the results of the interlaboratory study

		Number of negative results		Number of positive results	
Contamination level	**Number of results reported**	**REF**	**ALT**	**REF**	**ALT**
0	80	80	80	0	0
1	80	4	5	76	75
2	80	0	0	80	80

Table 6 Cross table of the results

		Reference Method		
		+	-	Sum
Alternative Method	+	**75 (PA)**	**0 (PD)**	146
	-	**1 (ND)**	**84 (NA)**	94
	Sum	147	93	240

- Relative sensitivity: 97%
- Relative specificity: 100%
- Relative accuracy: 100%
- Kappa: 0.99
- Acceptance Limit: ND-PD=1 (AL = 3) and ND+PD=1 (AL= 4)

Thus, the interlaboratory study showed no statistical difference between the results obtained by the two methods.

CONCLUSION:

According to the comparison and the collaborative study no statistical differences were found between the TRANSIA® PLATE *Salmonella* Gold and the reference method, EN ISO 6579 for the detection of *Salmonella* spp in foods, feeds and environmental samples.