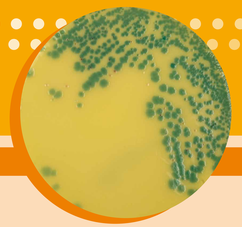
EVALUATION OF IBISA®, A NEW SELECTIVE AND CHROMOGENIC METHOD FOR THE DETECTION OF *SALMONELLA* SPECIES IN FOOD SAMPLES



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ABSTRACT

Introduction:

The use of chromogenic media for *Salmonella*'s detection in food samples has been described in different commercial methods. Nevertheless, most of these media based on the detection of C8-esterase activity only were reported to miss some strains such as S. Dublin or S. Bongori which express a weak enzymatic activity.

Purpose:

The objective of this study was to evaluate the ability of the IBISA® Method to detect *Salmonella* in naturally or artificially contaminated foods and in pure culture against EN ISO 6579 standard using XLD and conventional C8-esterase based chromogenic agar plate as screening media.

Method:

Food samples were 1/10 diluted in Buffered Peptone Water, then supplemented with ISS (IBISA® Specific Supplement) and incubated 16-20h at 41.5°C. 10 µl of the enriched broth were streaked on IBISA® plate and incubated 24 +/-3h at 37°C. On this new medium, *Salmonella* grow as typical large green colonies due to a specific mix of chromogenic esterase substrates.

An inclusivity study was performed with 90 strains of *Salmonella* including lactose positive strains, S. Dublin and non motile *Salmonella*. The exclusivity study was carried out on 35 non *Salmonella* strains.

174 food samples including naturally contaminated were tested according to the EN ISO 6579 reference method and IBISA® Method. Relative Accuracy, sensitivity and specificity were calculated during this evaluation.

Results:

The inclusivity and exclusivity study have shown a perfect ability of the IBISA to detect all *Salmonella* and to discriminate Non-*Salmonella* strains. In the meantime, XLD or conventional chromogenic medium demonstrated some difficulties to recover atypical strains such as *S.* Seftenberg and *S.* Dublin. On the 174 food samples, 55 were found positive and 116 were negative with both, IBISA® and NF EN ISO 6579 methods. 3 additional positive samples were detected and confirmed positive only with IBISA® method.

Significance:

In these trials, 48 hours IBISA® protocol showed comparable and even better sensitivity in comparison to the reference method for *Salmonella* detection in a variety of foods. Moreover the use of an esterase substrates mix allows IBISA® to detect *Salmonella* strains which are usually difficult to recover on classical chromogenic media

Introduction

The use of chromogenic media for *Salmonella*'s detection in food samples has been described in different commercial methods. Nevertheless, most of these media only based on the C8-esterase activity were reported to miss some strains such as *Salmonella enterica* ser. Dublin or *Salmonella Bongori* which express a weak enzymatic activity.

IBISA® medium is used for the detection of *Salmonella* spp in human foodstuffs and animal feeding stuffs and in environmental samples of food production. IBISA® principle lies on the use of a mix of chromogenic substrates (esterase activities) cleaved specifically by *Salmonella* and on the simultaneous detection of the β-glucosidase activity, allowing a differentiation of *Salmonella* among other Enterobacteriaceae. After incubation, *Salmonella* spp grow as very characteristic green colonies whereas other micro-organisms not inhibited are either colourless or magenta.

The objective of this study was to evaluate the ability of the IBISA® Method to detect *Salmonella* in pure culture by testing inclusivity and exclusivity. The aim was also to compare EN ISO 6579 standard using XLD and conventional C8-esterase based chromogenic agar plates as screening media and IBISA® with naturally or artificially contaminated food sample.

Method

• Inclusivity and Exclusivity

90 strains of *Salmonella* were tested following the IBISA® Protocol including the selective enrichment step to evaluate the inclusivity and 35 non-*Salmonella* strains were directly streaked on IBISA® from a TSB overnight culture to challenge the exclusivity.

The *Salmonella* strains tested during this study were selected for their isolation frequency and for their low C8-esterase activity described in the literature.

IBISA® plates were then incubated 24h +/-3h at 37°C +/- 1°C.

• Method Comparison

A comparison between the IBISA® method and the EN ISO 6579 reference method was carried out on more than 174 samples issued from different food categories:

Sample Catégories	Number of tested Samples
Chicken, Turkey, poultry	43
Raw beef	22
Raw milk cheese	37
Egg product	45
Prepared Meal	27
TOTAL	174

21 samples were naturally contaminated. The other positive samples were artificially contaminated by using a low level (<10 CFU/ 25g) of different stressed *Salmonella* strains. The stress intensity was evaluated by calculating the logarithmic difference between a non selective agar (TSA) and a selective one (XLD).

The relative accuracy, sensitivity, and specificity were calculated for both IBISA® method and the EN ISO 6579 reference method according to the EN ISO 16140 specifications.

Results

• Inclusivity and exclusivity

Fig 4a : IBISA® Exclusivity evaluation

Strains	Origin	IBISA Results	
		Growth	Color
Salmonella abortus ovis	Vet lab	+++	Green colonies
Salmonella annatum	Food Industry	+++	Green colonies
Salmonella arizonae	Duck	+++	Green colonies
Salmonella arizonae	Duck	+++	Green colonies
Salmonella arizonae	Food Industry	+++	Green colonies
Salmonella arizonae	Food Industry	+++	Green colonies
Salmonella bongori	ATCC 43975	+++	Green colonies
Salmonella bongori	Food Industry	+++	Green colonies
Salmonella brandenup	Food Industry	+++	Green colonies
Salmonella brandenburg	Milk	+++	Green colonies
Salmonella bredeney	Raw turkey	+++	Green colonies
Salmonella ceny	flour rabbit	+++	Green colonies
Salmonella Choleraesuis	Food Industry	+++	Green colonies
Salmonella Choleraesuis	ATCC 7001	++	Green colonies
Salmonella Choleraesuis	ATCC 10708	+	Green colonies
Salmonella Derby	food Industry	+++	Green colonies
Salmonella Derby	chicken carcass	++	Green colonies
Salmonella diarizonae	wheat semolina	+++	Green colonies
Salmonella diarizonae	food industry	+++	Green colonies
Salmonella diarizonae	CIP 82.31	+++	Green colonies
Salmonella dublin	Food Industry	+++	Green colonies
Salmonella dublin	Food industry	+++	Green colonies
Salmonella dublin	Milk product	+++	Green colonies
Salmonella dublin	Meat	+++	Green colonies
Salmonella dublin	Meat	+++	Green colonies
Salmonella dublin	Cattle	+++	Green colonies
Salmonella dublin	Meat	+++	Green colonies
Salmonella dublin	Cattle	+++	Green colonies
Salmonella dublin	Cattle	+++	Green colonies
Salmonella dublin	Poultry	+++	Green colonies
Salmonella dublin	Clinical	+++	Green colonies
Salmonella dublin	Cattle	+++	Green colonies
Salmonella dublin	Clinical	+++	Green colonies
Salmonella dublin	Milk product	+++	Green colonies
Salmonella dublin	Clinical	+++	Green colonies
Salmonella dublin	Cattle	+++	Green colonies
Salmonella dublin	Dairy industry	+++	Green colonies
Salmonella dublin	Milk product	+++	Green colonies
Salmonella dublin	Pig	+++	Green colonies
Salmonella dublin (monophasic)	Food Industry	+++	Green colonies
Salmonella dublin (monophasic)	Food Industry	+++	Green colonies
Salmonella enteritidis	Cake	+++	Green colonies
Salmonella enteritidis	Poultry Environment	+++	Green colonies
Salmonella enteritidis	Food industry	+++	Green colonies
Salmonella gallinarum	Food Industry	+	Green colonies

+ : Growth with small colonies ++ : Growth +++: Luxuriant growth

Strains	Origin	IBISA Results	
		Growth	Color
Salmonella gallinarum	Food Industry	+	Green colonies
Salmonella hadar	Vet lab	+++	Green colonies
Salmonella havana	Food Industry Environment	+++	Green colonies
Salmonella heidelberg	beef meat	+++	Green colonies
Salmonella indiana	Food Industry	+++	Green colonies
Salmonella infantis	Food Industry	+++	Green colonies
Salmonella infantis	Food Industry	+	Green colonies
Salmonella kedougou	Animal flour	+++	Green colonies
Salmonella kottbus	Food Industry Environment	+++	Green colonies
Salmonella livingston	Chicken carcass	+++	Green colonies
Salmonella london	Goat milk	++	Green colonies
Salmonella manhattan	beef meat	+++	Green colonies
Salmonella mbandaka	chicken carcass	+++	Green colonies
Salmonella montevideo	Raw beef	+++	Green colonies
Salmonella napoli	Food Industry	+++	Green colonies
Salmonella newport	Food Industry	+++	Green colonies
Salmonella orion	Food Industry	+++	Green colonies
Salmonella paratyphi A	SGSC 2690	++	Green colonies
Salmonella paratyphi A	CIP 55.40	++	Green colonies
Salmonella paratyphi A	CIP 54100	+++	Green colonies
Salmonella Paratyphi A	Clinical	+++	Green colonies
Salmonella Paratyphi A	CIP 55.39	+++	Green colonies
Salmonella Paratyphi A	CIP 55.38	++	Green colonies
Salmonella Paratyphi B	Clinical	+++	Green colonies
Salmonella Paratyphi B	Clinical	+++	Green colonies
Salmonella Paratyphi B	CIP 54.105	+++	Green colonies
Salmonella paratyphi C	CIP 55.108	+	Green colonies
Salmonella poona	NCTC4840	+++	Green colonies
Salmonella reading	Duck	+++	Green colonies
Salmonella rissen	food industry environment	+++	Green colonies
Salmonella saint paul	Food Industry	+++	Green colonies
Salmonella salamae	CIP 105631	+++	Green colonies
Salmonella schwarzengrund	Food Industry	+++	Green colonies
Salmonella seftenberg	chicken carcass	+++	Green colonies
Salmonella seftenberg	Milk product	+++	Green colonies
Salmonella Typhi	CIP54136	+++	Green colonies
Salmonella Typhi	Clinical	+	Green colonies
Salmonella Typhi	Clinical	++	Green colonies
Salmonella Typhi	CIP 54118	+	Green colonies
Salmonella Typhi	CIP 54137	+	Green colonies
Salmonella typhimurium	Food	+++	Green colonies
Salmonella typhimurium	S1 1,4,[5],12:- (tiramisu)	+++	Green colonies
Salmonella typhimurium	S1 1,4,[5],12:- (turquey)	+++	Green colonies
Salmonella typhimurium	ATCC 14028	+++	Green colonies
Salmonella virchow	food industry	+++	Green colonies

+ : Growth with small colonies ++ : Growth +++: Luxuriant growth

This inclusivity study demonstrates the ability of the IBISA® to detect 90 different *Salmonella* strains including lactose positive, non-motile strains but also *Salmonella Bongori* and *Salmonella* Dublin. On conventional C8-esterase based chromogenic medium, these last strains are known to express weakly their enzymatic activity and to be very difficult to detect. On IBISA®, all the tested strains appear typical with typical large green colonies after 24h of incubation including the 20 *Salmonella* Dublin isolated from different food samples. These performances are linked to the use of a specific chromogenic mix and a proprietary culture base formulation in IBISA® plates.

Fig 4b : IBISA® Exclusivity evaluation

Strains	IBISA®		
	Growth	Typicity on IBISA® plates	Colonies Color
Aeromonas hydrophila	-	N/A	N/A
Aeromonas hydrophila	-	N/A	N/A
Aeromonas hydrophila	-	N/A	N/A
Aeromonas hydrophila	-	N/A	N/A
Aeromonas hydrophila	-	N/A	N/A
Citrobacter diversus	-	N/A	N/A
Citrobacter diversus	+	Non typical	Pink
Citrobacter freundii	+++	Non typical	Pink
Citrobacter freundii	+	Non typical	Pink
Citrobacter koseri	-	N/A	N/A
Citrobacter koseri	-	N/A	N/A
Enterobacter aerogenes	++	Non typical	violet
Enterobacter cloacae	+++	Non typical	violet
Enterobacter sakazakii	-	N/A	N/A
Enterobacter sakazakii	++	Non typical	Violet
Escherichia coli	-	N/A	N/A
Escherichia coli	-	N/A	N/A
Escherichia hermanii	++	Non typical	White
Escherichia vulneris	-	N/A	N/A
Hafnia alvei	-	N/A	N/A
Klebsiella oxytoca	++	Non typical	Pink
Klebsiella pneumoniae	+++	Non typical	violet
Pantoea agglomerans	++	Non typical	violet
Pantoea agglomerans	-	N/A	N/A
Proteus mirabilis	-	N/A	N/A
Proteus vulgaris	-	N/A	N/A
Providencia spp	+++	Non typical	White
Pseudomonas aeruginosa	-	N/A	N/A
Serratia ficaria	-	N/A	N/A
Serratia fonticola	-	N/A	N/A
Serratia marcescens	+++	Non typical	Pink
Serratia marcescens	++	Non typical	Pink
Shigella flexneri	++	Non typical	White
Shigella sonnei	+++	Non typical	Pink
Yersinia enterocolitica	++	Non typical	White

The exclusivity study shows a very good selectivity of the IBISA® plates due to different antibiotics and selective agent included in the formulation. Moreover, the addition of a specific chromogenic substrate for the beta-glucosidase detection allows discriminating easily the other enterobacteriaceae from the *Salmonella* strains.

- : Inhibition
+ : Growth with small colonies
++ : Growth
+++: Luxuriant growth

• Method Comparison

Fig 5 : Comparison of the accuracy of IBISA® Method and Iso 6579 reference • Method on 174 food samples

	EN ISO 6579 Positive Results	EN ISO 6579 Negative Results
IBISA® Positive Results	55	3+
IBISA® Positive Results	0	116

55 samples were confirmed positive with *Salmonella* by both methods. 3 food samples were only found positive on IBISA® and negative with the ISO mandatory method.

The interfering flora on these 3 samples was very important and the interpretation of the XLD and ASAP media was impossible. On the contrary, the high selectivity of the IBISA® specific Supplement (ISS) in the Buffer Peptone Water and the characteristic size and green coloration of *Salmonella* colonies on IBISA® plates allow easy detection of the typical colonies.

Fig 6 : Calculation of relative Accuracy, Sensitivity and Specificity of IBISA® Method against the EN ISO 6579 Reference Method

	PA	NA	ND	PD	SUM N	Relative Accuracy AC (%) [100x(PA+NA)]/N	N+ PA + ND	Relative sensitivity SE (%) [100xPA]/N+	N- NA + PD	Relative specificity SP (%) [100xNA]/N-
TOTAL	55	116	0	3	174	98.2	55	100	119	97.4

This study allows concluding to excellent performances of IBISA® method versus the reference method for *Salmonella* detection in food samples. In such calculation, the EN ISO 6579 is considered to be the gold standard, but due to the fact that IBISA® method have detected 3 additional confirmed positive sample, the specificity could be recalculated to 100 %.

Conclusion

This study demonstrated the performances of IBISA®, a novel chromogenic method for the detection of *Salmonella* spp in foodstuffs and environmental samples.

The combination of its high selectivity and its specific enzymatic detection system improves the sensitivity of the method and allows the detection of strains as *Salmonella* Dublin or *Salmonella bongori* compared to classical C8-esterase based chromogenic medium.

IBISA® is a reliable alternative method for the detection of *Salmonella* spp. It is perfectly adapted to laboratory workflow since it is a simple and accurate method that allows to easily screen negative samples within 48 hours.

Fig 1 : IBISA® Protocol for the detection of Salmonella spp and the different confirmation tests possibilities.

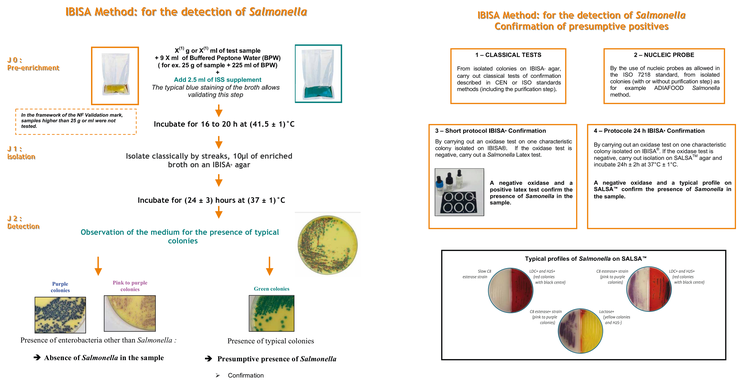
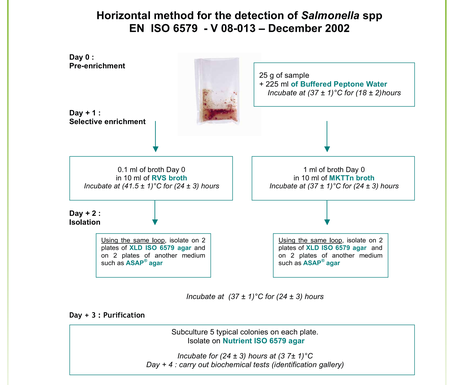


Fig 2 : EN ISO 6579 : Horizontal method for the detection of Salmonella spp



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