

Performance of the RambaQUICK™ Salmonella method

Use of CHROMagar™ Salmonella Plus and RambaQUICK™ Salmonella for detection and isolation of Salmonella species including lactose positive strains

Laboratory

CHROMagar SAS
29 avenue George Sand
93210 La Plaine St-Denis
FRANCE

This report contains 16 pages, including 2 pages of annexes

Table of Contents

1. Introduction	3
2. Performance of the RambaQUICK™ Salmonella formula.....	4
2.1. Analytical data	4
2.2. Enrichment/Selectivity evaluation.....	5
2.3. Sensitivity evaluation with a foodstuff.....	7
2.4. Confirmatory biochemical and serological testing.....	7
3. Independent laboratory evaluation of the product	8
3.1. Comparison to the ISO 6579 method	8
3.2. Comparison to Rappaport-Vassiliadis medium.....	11
4. Conclusion.....	13
5. Literature	14
Annexes.....	15
Annex 1. Website information about RambaQUICK™ Salmonella	15
Annex 2. Website information about CHROMagar™ Salmonella Plus.....	16

1. Introduction

Salmonella species are known pathogenic agents behind major worldwide foodborne diseases and epidemiologic issues. The ISO 6579 is the bacteriological standard method for the detection of *Salmonella* spp. (1), but the occurrence of lactose-positive *Salmonella* interferes with the recognition of suspect colonies. Accurate and reliable methods for the detection of *Salmonella* are necessary to ensure public health safety and regulatory compliance.

CHROMagar™ Salmonella Plus (ref. SA16) has been developed to enable clear and straightforward visible detection of *Salmonella* spp., including lactose-positive *Salmonella*, *S. Typhi*, *S. Paratyphi* and serovars (2 and Fig. 1) in food, water, feed and environmental samples. ISO 6579 (2017) includes CHROMagar™ Salmonella Plus as a choice of a second selective plating medium (which is complementary to Xylose Lysine Deoxycholate, XLD agar) meeting the standard's requirement for the detection of lactose-positive *Salmonella*. The best functions found in conventional enrichment broths, namely Rappaport-Vassiliadis medium with soy (RVS broth) and Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn), are achieved by using a shorter enrichment step with only RambaQUICK™ Salmonella selective broth (ref. SQ001). After the revivification phase in Buffered Peptone Water (BPW), bacteria are incubated at 41.5°C for 7 hours (instead of 24 hours as per ISO 6579) in RambaQUICK™ Salmonella. During this selective enrichment, *Salmonella* bacteria multiply in an exponential growth phase and competitive flora are inhibited. Finally plating is carried out on the same day (see annexes 1 & 2).

CHROMagar™ Salmonella Plus consists of a powder base and a liquid supplement, which are stored at 15-30°C. Optionally, plates can have an opaque background when a white opaque supplement is incorporated during their preparation. Samples can be streaked onto agar plates after selective broth enrichment employing RambaQUICK™ Salmonella which is composed of a powder base (see annex 1). Medium plates are incubated under aerobic conditions at 37 °C for 18-24 hours. *Salmonella*, including lactose-positive *Salmonella* species are distinctively grown as mauve colonies while *E. coli* and some *Proteus* species grow as colourless colonies, and coliforms grow blue colonies on CHROMagar™ Salmonella Plus.

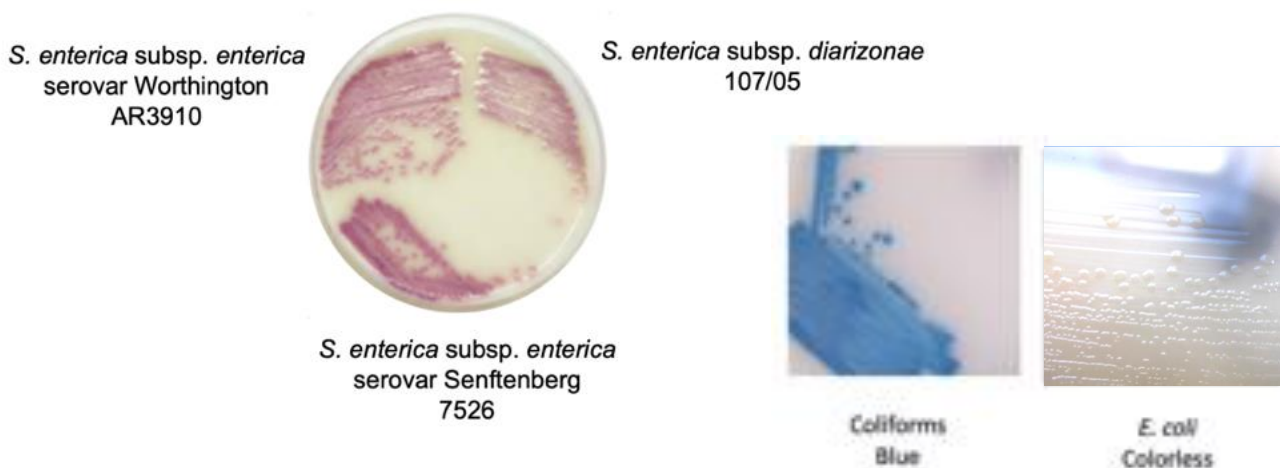


Figure 1. Detection of *Salmonella* strains (ONPG⁺, lactose⁺) on CHROMagar™ Salmonella Plus (2).

Together, CHROMagar™ Salmonella Plus (one culture plate) and RambaQUICK™ Salmonella (one selective enrichment broth), referred to here as the **RambaQUICK™ Salmonella method**, provide a powerful solution to the ever-present challenge of *Salmonella* detection, ensuring that safety and quality standards are met with confidence and efficiency.

This document compiles the **RambaQUICK™ Salmonella method** evaluations at two stages:

- In-house evaluations of the selective enrichment formula with pure strains and with food samples artificially contaminated. Biochemical and serological confirmatory testing on strains cultured in the broth formula and grown on CHROMagar™ Salmonella Plus plates.
- Independent laboratory evaluations using *Salmonella* strains and food samples.

2. Performance of the RambaQUICK™ Salmonella formula

2.1. Analytical data

Different *Salmonella* and other bacterial strains (n=30 and n=23, respectively) were incubated in RambaQUICK™ Salmonella for 7 hours at 41.5°C and streaked on CHROMagar™ Salmonella Plus. Agar plates were incubated for 18-24 hours at 37°C to evaluate the inclusivity and exclusivity of the formula in the **RambaQUICK™ Salmonella method**. Results are shown in Tables I and II.

Table I. inclusivity **RambaQUICK™ Salmonella method**.

<i>Salmonella</i> strains	Strain #	Colonie appearance	<i>Salmonella</i> strains	Strain #	Colonie appearance
<i>Salmonella</i>	AR3011	M++, 2	<i>S. Senftenberg</i> (lac+)	AR5093	M+, 2.5
<i>S. Typhimurium</i>	AR3015	M+, 1	<i>S. Abony</i>	CIP 110658	M+, 1.5
<i>S. Typhimurium</i>	ATCC® 13311	M++, 1.5	<i>S. Gallinarum</i>	CIP 57.53	M+/-, 0.6-1
<i>S. Typhimurium</i>	ATCC® 14028	M++, 2	<i>S. Kentucky</i>	CIP 105623	M++, 2
<i>S. Enteritidis</i>	ATCC® 13076	M+, 1	<i>S. indica</i>	ATCC® 43976	M-/+ , 2
<i>S. Typhi</i>	AR3104	M, 1	<i>S. Abaetetuba</i>	ATCC® 35640	M+/-, 3
<i>S. Typhi</i>	AR3105	M+, 2	<i>S. Paratyphi A</i>	AR5082	M++, 2
<i>S. Typhi</i>	AR4052	M+, 1	<i>S. Paratyphi A</i>	CIP 55.39	M++, 2
<i>Salmonella</i> spp.	AR4053	M++, 3	<i>S. Paratyphi B</i>	AR5086	M++, 2
<i>S. Dublin</i>	AR3580	Uncol., 3	<i>Salmonella</i> spp.	AR5149	M++, 2
<i>S. arizonae</i> (lac+)	AR3910	M, 1	<i>Salmonella</i> spp.	AR5150	M++, 2
<i>S. arizonae</i>	ATCC® 13314	M+, 2.5	<i>Salmonella</i> spp.	AR5151	M++, 2
<i>S. Hadar</i>	AR4125	M+, 2	<i>Salmonella</i> spp.	AR5424	M+, 1
<i>S. St Paul</i>	AR4316	M++, 3	<i>Salmonella</i> spp.	AR5425	M+, 1
<i>S. Derby</i>	AR4429	M+, 2	<i>Salmonella</i> spp.	AR5430	M++, 1.5

M, mauve ; *B*, blue ; *Uncol.*, uncolored ; +, color intensity ; colony size in mm ; AR, CHROMagar™ strain collection ; ATCC, American Type Culture Collection ; CIP, Strain collection Institut Pasteur.

Table II. Exclusivity of RambaQUICK™ Salmonella method.

Strains	Strain #	Colonie appearance
<i>Citrobacter freundii</i> [§]	ATCC® 8090	B++, 1
<i>Citrobacter</i> spp. [§]	AR3378	B++, 1.5
<i>C. freundii</i> [§]	AR3870	B++, 1
<i>Escherichia coli</i>	ATCC® 25922	Uncol., 2
<i>E. coli</i>	AR3740	Uncol., 2
<i>E. coli</i>	AR5428	Uncol., 2
<i>E. coli</i>	AR3859	Uncol., 2
<i>E. coli</i>	AR3741	Uncol., 1.5
<i>E. coli</i>	AR3857	Uncol., 1.5
<i>E. coli</i>	AR5011	Uncol., 2
<i>E. coli</i>	AR5427	M--/+, 2
<i>E. coli</i>	AR5428	Uncol., 2
<i>E. hermanii</i>	AR5849	Uncol. DZ
<i>Klebsiella oxytoca</i>	AR5755	B++, 3
<i>Hafnia alvei</i>	AR3862	-
<i>Hafnia</i> spp.	AR5010	M--/+, 3 [¶]
<i>Morganella morganii</i>	AR4080	-
<i>Proteus mirabilis</i>	AR3022	-
<i>Serratia marcescens</i>	AR5569	-
<i>Staphylococcus aureus</i>	ATCC® 25923	-
<i>S. aureus</i>	AR3916	-
<i>Pseudomonas aeruginosa</i>	ATCC® 9027	-
<i>P. aeruginosa</i>	ATCC® 10145	-

M, mauve ; *B*, blue ; *Uncol.*, uncolored ; +, color intensity ; colony size in mm ; *DZ*, dense zone ; -, absence of growth (but growth on TSA) ; *AR*, CHROMagar™ strain collection ; *ATCC*, American Type Culture Collection. [§]*Citrobacter* is a false positive on XLD agar. [¶]Colonies show weaker coloration than *Salmonella* colonies, not considered a false positive.

2.2. Enrichment/Selectivity evaluation

Mixtures of bacteria species, *Salmonella* (AR4053, AR3011, AR3015, AR4052, AR3104 and AR3910 (*lac+*) strains at 10⁻⁷ dilutions), *E. coli* (AR3741, AR3859 and AR3857 strains at 10⁻³ dilutions) and *Citrobacter* (AR3870, ATCC®8090 and AR3378 strains at 10⁻³ dilutions) were used to evaluate the RambaQUICK™ Salmonella selectivity. A sample of 0.1 mL of each of these mixes was added to a BPW tube to incubate 18 hours at 37°C. An aliquot of 0.1 mL was inoculated into a RambaQUICK™ Salmonella tube to incubate for 7 hours at 41.5°C. Plating of a 10 µL aliquot of the BPW culture and the enriched RambaQUICK™ Salmonella broth (Time 7h) was carried out on CHROMagar™ Salmonella

Plus plates. The selective *Salmonella* enrichment results in the detection of either some mauve colonies in a mix of blue and colourless colonies or the presence of 90-100 % of mauve colonies and less than 10 % of blue and colourless colonies on CHROMagar™ Salmonella Plus plates (Fig. 2).

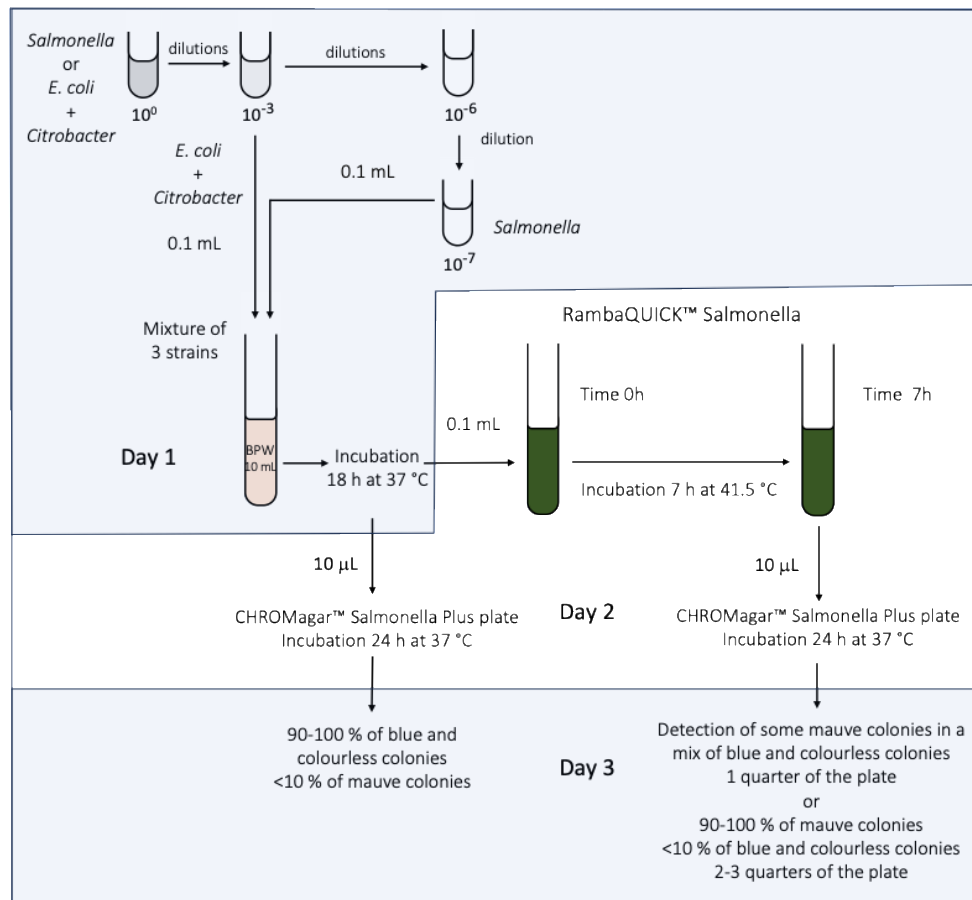


Figure 2. Selectivity evaluation of RambaQUICK™ Salmonella.

Combinations of bacterial strains of interest used in the **RambaQUICK™ Salmonella method** demonstrated the enrichment and chromogenic differentiation of *Salmonella* (Table III).

Table III. Examples of results obtained with the **RambaQUICK™ Salmonella method** with different strains.

Mixture N°	Species	Strain	Dilution of inoculum	% of colonies		Colony aspect
				At time 0 h	At time 7 h	
1	<i>Salmonella</i> spp.	AR4053	10 ⁻⁷	ND	~90	mauve
	<i>E. coli</i>	AR3741	10 ⁻³	~60	< 5	colourless
	<i>C. freundii</i>	AR3870	10 ⁻³	~40	< 5	blue
2	<i>Salmonella</i>	AR3011	10 ⁻⁷	~2	~90	mauve
	<i>E. coli</i>	AR3859	10 ⁻³	~50	ND	colourless
	<i>C. freundii</i>	ATCC®8090	10 ⁻³	~50	~10	blue
3	<i>S. Typhimurium</i>	AR3015	10 ⁻⁷	~2	~90	mauve
	<i>E. coli</i>	AR3857	10 ⁻³	~50	ND	colourless
	<i>Citrobacter</i> spp.	AR3378	10 ⁻³	~50	~10	blue

AR, CHROMagar™ strain collection ; ATCC, American Type Culture Collection ; ND, non-detected.

2.3. Sensitivity evaluation with a foodstuff

The detection of *Salmonella* in food products, such as cocoa, is critical due to the complexity of chocolate as a polyphenol-rich matrix which presents unique challenges for microbiological analysis. Rigorous protocols involving key steps such as sample preparation, enrichment, selective inoculation and confirmation by biochemical and molecular methods are required.

A cocoa sample was used as an illustrative use case, to evaluate the sensitivity of the formula. Ten grammes of cocoa containing less than 1000 UFC/g were tested without (n=2) or with *Salmonella* spp. (AR4053 strain, n=4) or *E. coli* (AR3741, n=4) spiking at approximately 1000 UFC/g. A revivification step in BPW during 24 h at 37°C, followed by the inoculation of 0.1 mL of this pre-enrichment was performed to follow the ISO 6579 method. Also, 0.1 mL of pre-enriched samples were inoculated into 10 mL of RambaQUICK™ *Salmonella* selective broth. After a 7 hour incubation at 41.5°C, 10 µL of these tubes were seeded onto CHROMagar™ *Salmonella* Plus and onto XLD agar plates to be incubated at 37°C for 18-24 hours.

E. coli colonies grew white on CHROMagar™ *Salmonella* Plus and yellow on XLD agar. *Salmonella* was successfully detected as mauve colonies with the **RambaQUICK™ *Salmonella* method** (black colonies grown on XLD agar) in the four AR4053-spiked samples tested, offering a streamlined workflow that delivered results in a fraction of the time required by the ISO 6579 method.

2.4. Confirmatory biochemical and serological testing

Confirmation of bacterial enrichment in RambaQUICK™ *Salmonella* using biochemical and serological testing can be performed directly from isolated colonies grown on CHROMagar™ *Salmonella* Plus (Tables IV and V). These tests can overcome the limitations of the method and provide a serotyping procedure for unknown *Salmonella* isolates.

- **Oxidase test.** Performed on Filter Paper (Whatman No. 1 or equivalent) with a few drops of Oxidase test reagent or by streaking a loopful of bacteria onto the reagent-saturated paper with a platinum loop or wooden applicator stick. Positive reactions turn the bacteria violet to purple immediately (*e.g.* some *Pseudomonas* showing a mauve colouration), or up to 30 s. Negative reactions remain colorless or turn light-pink/light-purple after 30 sec (Beckton Dickinson, ref. 261181).
- **ONPG test.** Performed with reagent disc added to a bacterial suspension in sterile saline water and incubated 2 hours at 37 °C. Positive reactions turn the bacterial suspension yellow as is the case for *E. coli* and *Salmonella* lactose (+) strains (Biomérieux, ref. 55601).
- **Urea-Indole test.** Performed by the inoculation of the yellow-orange medium and incubation for 2 hours at 37°C. Urease positive bacteria (*e.g.*, *Proteus*) turn liquid media red-brown. The degradation of L-tryptophane contained in the broth is detected by the presence of a pink-red ring in the surface when the James reactant is added. This results in an indole positive test, as for *E. coli* (Biomérieux, ref. 55752).
- **Serological tests.** Performed on suspected colonies grown on CHROMagar™ *Salmonella* Plus can confirm the presence of *Salmonella* strains (*Salmonella* confirm latex kit, Bio-Rad ref. 355-6711; Microgen *Salmonella* latex test), *S. Typhi* and *S. Paratyphi* (Antiserum *Salmonella* Vi monovalent, Bio-Rad ref. 3560951B) as well as *Salmonella* serovars (*Salmonella* Polyvalent O Groupe A-S Agglutination serum, Oxoid ref. ZCO2/R30858201).

Table IV. Biochemical testing of bacterial colonies on CHROMagar™ Salmonella Plus.

Species	Strain	Oxidase test	ONPG test	Urea test	Indole test
<i>S. Typhi</i>	AR4052	-	-	-	-
<i>Salmonella</i> spp.	AR4053	-	-	-	-
<i>S. arizonae</i> (lac+)	AR3910	-	+	-	-
<i>E. coli</i>	AR3740	-	+	-	+
<i>E. coli</i>	AR3859	-	+	-	+
<i>Citrobacter</i> spp.	AR3378	-	+	-	-
<i>Citrobacter koseri</i>	AR3134	-	+	-	+
<i>Proteus mirabilis</i>	AR3022	-	-	+	-
<i>P. aeruginosa</i>	ATCC® 9027	+	-	-	-

+, positive enzymatic reaction test ; -, negative enzymatic reaction test.

Table V. Serological testing of bacterial colonies on CHROMagar™ Salmonella Plus.

Species	Strain	Salmonella confirm latex kit	Antiserum Salmonella Vi monovalent	Polyvalent O Groupe A-S Agglutinating Serum
<i>S. Typhi</i>	AR4052	-	+	+/-
<i>S. Typhimurium</i>	ATCC® 13311	+/-	-	+/-
<i>S. Typhimurium</i>	ATCC® 14028	+	-	+/-
<i>S. Abaetetuba</i>	ATCC® 35640	+	-	+
<i>S. Dublin</i>	AR3580	+	-	+
<i>S. arizonae</i> (lac+)	AR3910	+/-	-	+
<i>S. Abony</i>	CIP 110658	+	-	+
<i>E. coli</i>	AR3859	-	/	/
<i>Hafnia alvei</i>	AR3862	-	/	/
<i>Citrobacter</i> spp.	AR3378	-	/	/

+, positive serological test ; -, negative serological test ; +/-, slow serological reaction ; /, test not performed.

Moreover, a study using Microgen Salmonella latex test and CHROMagar™ Salmonella Plus showed 100% specificity and 100% sensitivity using 111 *Salmonella* strains and 35 non-target bacterial strains, respectively (3).

3. Independent laboratory evaluation of the product

3.1. Comparison to the ISO 6579 method

Naturally and artificially contaminated food samples were tested using both the **RambaQUICK™ Salmonella method** and ISO 6579 by independent laboratories.

- a) Laboratory Adria Normandie (4-6). Foodstuffs tested (n=64), which were known to be either uncontaminated or contaminated with *Salmonella* were meat products, seafood, egg products, pastries, and dairy products. Other media plates employed were XLD and Rambach agar. The data from this study showed agreement between the two methods regarding the absence of *Salmonella* detection in 39 samples. *Salmonella* was detected in the other 25 positive samples with the **RambaQUICK™ Salmonella method** whereas only 24 samples resulted positive with the ISO 6579 method, as shown in Table VI.

Table VI. Comparison of the performance of the ISO 6579 and the **RambaQUICK™ Salmonella method** (Laboratory Adria Normandie).

# sample	Food sample (Sample ID)	ISO 6579 method	RambaQUICK™ Salmonella method
1	Egg 412	+	+
2	Egg 512	+	+
3	Duck liver	-	+
4	Sausages	+	+
5	Meat	+	+
6	Ham with bone	-	-
7	Pork ear	+	+
8	Scallops	+	+
9	Pork liver	+	+
10	Pork liver	+	+
11	Raw milk	+	+
12	Raw cream	+	+
13	Peeled prawns	+	+
14	Peeled prawns	+	+
15	Pudding flan	+	+
16	Minced meat	+	+
17	Salmon dish	+	+
18	Egg	+	+
19	Egg	+	+
20	Hakefish (21)	-	-
21	Minced meat (22)	-	-
22	Chicken (23)	-	-
23	Minced steak (24)	-	-
24	Cuttlefish (25)	-	-
25	Salmon lasagne (26)	-	-
26	Pudding flan (27)	-	-
27	Liver pâté (28)	-	-
28	Chicken (29)	-	-
29	Low salt ham (30)	-	-
30	Duck leg (31)	-	-
31	Egg 312 (32)	-	-
32	Egg 108 (33)	-	-

# sample	Food sample (Sample ID)	ISO 6579 method	RambaQUICK™ Salmonella method
33	Sausages (34)	-	-
34	Red mullet fish (35)	-	-
35	Pork throat (36)	-	-
36	Egg 312 4000G (37)	-	-
37	Minced meat (38)	-	-
38	Jellied pork tongue (39)	-	-
39	Garlic sausage (40)	-	-
40	Chicken leg (41)	-	-
41	Pork liver (42)	-	-
42	Pork throat (43)	-	-
43	Meat (44)	-	-
44	Raw cream (26)	-	-
45	Scallops (27)	-	-
46	Ham 6D (28)	+	+
47	Meat 4D (29)	-	-
48	Pork ear (30)	-	-
49	Pork liver (31)	+	+
50	Pork liver (32)	-	-
51	Ham with bone (33)	-	-
52	Ham with bone (34)	-	-
53	Pork throat (35)	-	-
54	Pork throat 12080010 (36)	-	-
55	Pork throat 1006016 (37)	-	-
56	Ham with bone 1206008 (38)	-	-
57	Pork ear 1117004 (39)	+	+
58	Pork ear 1117005 (40)	+	+
59	Pork head 0504043 (41)	+	+
60	Meat 4D 0504045 (42)	+	+
61	Ham 6D 1006019 (43)	-	-
62	Pork liver 1208015 (44)	+	+
63	Egg 312 4000D (45)	-	-
64	Egg 312 2000 (46)	-	-

Positive tests for ISO 6579 method: 24 (96 %)

Positive tests for RambaQUICK™ Salmonella method: 25 (100 %)

- b) A heat treatment (3.5 to 8 min at 57°C) intended to stress *Salmonella* strains was used before bacterial spiking foodstuffs at 1-30 UFC/25 g. Spiked *Salmonella* species including the *lac+* species could be detected as mauve colonies with the **RambaQUICK™ Salmonella method**, as shown in Table VII.

Table VII. Results obtained in the sensitivity evaluation of the ISO 6579 and the **RambaQUICK™ Salmonella method** with artificially contaminated foodstuffs (Laboratory Adria Normandie).

Food sample (Sample ID)	Spiked Salmonella species	ISO 6579 method	RambaQUICK™ Salmonella method
Raw milk (11)	<i>S. Virchow</i>	+	+ (mauve colonies)
Raw cream (12)	<i>S. enterica</i> serovar Senftenberg	+	+ (mauve colonies)
Peeled prawns (13)	<i>S. Typhimurium</i>	+	+ (mauve colonies)
Peeled prawns (14)	<i>S. enterica</i> serovar Senftenberg	+	+ (mauve colonies)
Pudding flan (15)	<i>S. enteritidis</i>	+	+ (mauve colonies)
Minced meat (16)	<i>S. Typhimurium</i>	+	+ (mauve colonies)
Salmon dish (17)	<i>S. enterica</i> serovar Derby	+	+ (mauve colonies)
Egg (18)	<i>S. enteritidis</i>	+	+ (mauve colonies)
Egg (19)	<i>S. enteritidis</i>	+	+ (mauve colonies)

In these studies, an optimal specificity was obtained with incubation conditions of the RambaQUICK™ Salmonella for 7 hours at 41.5°C (see annex 1).

- c) Laboratory ASEPT (7). Tested food samples previously known to be either uncontaminated or contaminated by *Salmonella* (n=26). In this study, negative samples proved to be consistent for both methods. Detection of *Salmonella* in ten out of eleven positive samples was possible using both methods (sensitivity 91%), as shown in Table VIII. However, the **RambaQUICK™ Salmonella method** gave faster results, two days earlier.

Table VIII. Results obtained in the sensitivity evaluation of the ISO 6579 and the **RambaQUICK™ Salmonella method** (Laboratory ASEPT).

# sample	Food sample	ISO 6579 method	RambaQUICK Salmonella method
19656	Duck foie gras	+	+
19657	Raw duck magret	-	-
19658	Raw duck magret	+	+
19659	Raw duck magret	-	-
19660	Smoked duck magret	-	-
19661	Raw duck magret	-	-
19662	Smoked duck magret	-	-
19663	Smoked duck magret	-	-
19664	Raw duck magret	-	-
19665	Raw duck magret	+	+
19666	Raw duck magret	+	+
19667	Raw duck magret	-	-
19668	Duck foie gras	-	-
19898	Whole Egg	-	-
19899	Egg white	-	-
19900	Whole Egg	-	-
19901	Egg white	-	-
19902	Egg white	+	+
19903	Whole Egg	+	+
19904	Whole Egg	+	+
19905	Egg yolk	+	-
19906	Egg yolk	+	+
19907	Egg yolk	+	+
19908	Pet food	-	-
19909	Pet food	-	-
19910	Pet food	-	+
Positive tests		10	10
%		91%	91%

3.2. Comparison to Rappaport-Vassiliadis medium

Furukawa and collaborators (8) used different bacterial strains to artificially contaminate retail chicken samples. Twenty-five grams of spiked food were incubated overnight at 35 °C in 225 mL of BPW. A pre-enrichment sample of 0.1 mL was inoculated into 10 mL of RambaQUICK™ Salmonella broth and incubated for 7 hours at 42 °C. A pre-enrichment sample of 0.5 mL was inoculated into 10 mL of RV medium (BPW-RV method). Samples of 10 µL of each broth culture were streaked on CHROMagar Salmonella medium. Agar plates were incubated for 18 hours at 35 °C before performing serological testing on mauve colonies.

Obtained data confirmed that RambaQUICK™ Salmonella supports the growth of *Salmonella* strains and inhibits the growth of non-target microorganisms tested. In contrast, an *Enterobacter cloacae* strain was not inhibited in the BPW-RV method (Table IX).

Table IX. Data obtained in the evaluation of RambaQUICK™ Salmonella.

Bacterial strains	Strain #	BPW-RV method	Method with RambaQUICK™ Salmonella
<i>S. Enteritidis</i>	001	+	+
<i>S. Enteritidis</i>	002	+	+
<i>S. Enteritidis</i>	003	+	+
<i>S. Enteritidis</i>	004	+	+
<i>S. Typhimurium</i>	ATCC® 13311	+	+
<i>Escherichia coli</i>	ATCC® 25922	-	-
<i>Citrobacter freundii</i>	ATCC® 8090	-	-
<i>Enterobacter cloacae</i>	ATCC® 13047	+	-
<i>Klebsiella pneumoniae</i>	NCTC 9636	-	-
<i>Proteus mirabilis</i>	ATCC® 29906	-	-
<i>Pseudomonas aeruginosa</i>	ATCC® 27853	-	-
<i>Enterococcus faecalis</i>	ATCC® 29212	-	-
<i>Staphylococcus aureus</i>	ATCC® 25923	-	-
<i>Bacillus subtilis</i>	ATCC® 6633	-	-
<i>Candida albicans</i>	ATCC® 10231	-	-

ATCC, American Type Culture Collection ; NCTC, National Collection of Type Cultures ; +, growth ; -, absence of growth.

Furthermore, 58 samples of retail chicken meats were analyzed. The detection rate within two days for the **RambaQUICK™ Salmonella method** was 63.8% (A total of 37 positive results), being higher than the detection rate for the BPW-RV method of 31.0% (A total of 18 positive results), colonies of non-target flora were detected in light pink and in blue (8, Table X). The authors concluded that the **RambaQUICK™ Salmonella method** was useful for the quality control workflow in the laboratory of food industry.

Table X. Frequency of organisms isolated from food using BPW-RV or RambaQUICK™ Salmonella methods.

Colony colour	Identified organism	Serotype	N° of isolates	
			BPW-RV method	RambaQUICK® Salmonella method
Maive	<i>Salmonella</i> spp.	O7	10	22
		O4	5	12
		O8	3	3
Light pink	<i>P. mirabilis</i>		1	1
Blue	<i>E. cloacae</i>		30	30
	<i>E. asburiae</i>		7	7
	<i>K. oxytoca</i>		17	17
	<i>C. koseri</i>		2	2
	<i>A. baumannii</i>		1	2

4. Conclusion

The performance of the **RambaQUICK™ Salmonella method** has been demonstrated by several evaluations which included inclusivity/exclusivity studies and sensitivity focused on the detection of *Salmonella* in diverse food samples.

Parameter	Performance of RambaQUICK™ Salmonella method (SQ001 & SA16)
Inclusivity / Exclusivity	97 % / 96 % with bacterial strains
Detection of <i>Salmonella</i> including lactose (+) strains	Sensitivity 91-100 % (5, 8) Specificity 100 % (4)
Morphological appearance of <i>Salmonella</i> colonies	Selective enrichment visible as distinctive mauve colonies after streaking or spreading on CHROMagar™ Salmonella Plus
Turnaround time	Food samples can be determined as negative in 2 days A positive sample can be determined in only 3 days
Biochemical ID confirmation and other tests	Tests: oxidase, urease, indole, serological MALDI-TOF MS (9) and PCR (10) directly from colony

The **RambaQUICK™ Salmonella method** meets ISO 6579-1:2017 requirements by detecting lactose positive *Salmonella*.

With proper storage at 15-30°C, the shelf life of RambaQUICK™ Salmonella powder base is 2 years. The shelf life of the RambaQUICK™ Salmonella broth distributed in tubes is 6 months when stored at 2-8 °C.

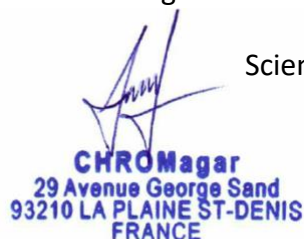
The results of the **RambaQUICK™ Salmonella method** are easy to read with the naked eye and a final identification on suspected colonies must be done by biochemical and serological tests (*i.e.*, latex agglutination) or by mass spectrometry or PCR. Testing can be carried-out directly on colonies grown on CHROMagar™ Salmonella Plus.

This medium has a very high sensitivity but some limitations can be pointed out:

- Some *S. enterica* serovar Dublin may appear colourless, nevertheless this serovar is rarely encountered.
- Some *E. coli* strains may develop a very slight mauve colouration but can be characterized by an indol test.
- Some *Pseudomonas* species may have similar mauve colony aspect but can be characterized by an oxidase test.

Hugo CRUZ RAMOS, PhD.

Scientific Expert




5. Literature

- 1) ISO 6579:2017. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella*.
- 2) de Beaumont, C., Breuil, J., Dedicova, D. and Tran, Q. 2006. Evaluation of a new chromogenic medium, CHROMagar™ Salmonella Plus, for the detection of *Salmonella* spp. Including lactose positive *Salmonella*, *S. Typhi* and *S. Paratyphi*. Poster P998, ECCMID.
- 3) Mugg, P. and Ukaegbu, K. 2016. Microgen Salmonella latex and CHROMagar™ Salmonella internal Assessment – Comparison reference cultures. 1-17 pp.
https://www.chromagar.com/fichiers/1487596052Microgen_Salmonella_Chromagar_Salmonella_Plus_report_Final_120416.pdf
- 4) Adria Normandie. 2006. Étude de la méthode « SalmoQuick ». Compte rendu. RED-06-105-1. 11 pp.
- 5) Adria Normandie. 2006. Étude de la méthode « SalmoQuick » modifiée. Compte rendu. RED-06-154-2. 11 pp.
- 6) Adria Normandie. 2006. Étude de la méthode « SalmoQuick » échantillons « challenging ». Compte rendu. RED-06-203-1. 6 pp.
- 7) ASEPT SAS. 2006. Essais préalables à une étude de validation. Méthode SalmoQuick. Document V2. 9 pp.
- 8) Furukawa, R., Sakairi, M., and Ishihara, Y. 2009. Evaluation of RambaQUICK Salmonella for Rapid Detection of *Salmonella* from Food Samples. *Jpn. J. Food Microbiol.* **26**: 177-182.
- 9) Lüthje, P., Pranada, A.B., Carruthers-Lay, D., Desjardins, M., Gaillot, O., Wareham, D. Clesielczuk, H. and Özenci, V. 2017. Identification of microorganisms grown on chromogenic media by MALDI-TOF MS. *J. Microbiol. Meth.* **136**: 17-20.
- 10) Ribeiro, S., Mourão, J., Rebelo, A., Novais, C., Peixe, L., and Antunes, P. 2019. Detection of *Salmonella* serotypes adapted to diverse stresses in poultry meat at the processing level in Portugal. Poster 1591. ECCMID.

Annexes


Annex 1. Website information about RambaQUICK™ Salmonella


[Our Company](#)
[Products](#)
[Technical documents](#)




The Chromogenic Media Pioneer

[Find your distributor](#)
[Contact](#)


EN





Clinical Microbiology
A complete range of culture media to help in clinical diagnosis




Food Industry
A complete range of culture media for the food industry



Water Testing
A complete range of culture media for water analysis



Veterinary Microbiology
A complete range of culture media for the veterinary sector



RambaQUICK™ Salmonella

For Rapid detection of *Salmonella* species including *S. typhi*, *S. paratyphi* and lactose positive *Salmonella*

[Order References](#)
Please use these references when contacting your local distributor :

To prepare 5000 mL of media (250 tests) : 3 packs of SQ001 + 1 pack of SA162

RambaQUICK™ Salmonella Method

- ① Pre-enrichment in buffer Peptone water for 18 h at 37 °C
- ② Transfer 0.1 mL of the pre-enrichment into 10 mL of RambaQUICK™ Salmonella broth and incubate for 7 h at 41.5 °C
- ③ Streak 10 µL of RambaQUICK™ in a CHROMagar™ Salmonella Plus plate and incubate 18-24 h at 37 °C

Reading and Confirmation

1: oxydase test
2: rapid urease test
3: serology

VS ISO standard method ISO 6579

- ① Pre-enrichment in buffer Peptone water for 18 h at 37 °C
- ② Transfer 0.1 mL into 10 mL of RVS broth and incubate for 24 h at 41.5 °C
- ③ Transfer 1 mL into 10 mL of MKTT broth and incubate for 24 h at 37 °C

④
XLD Plate

⑤
Second Plate

⑥
XLD Plate

⑦
Second Plate

Incubation 24 h at 37 °C

⑧
Nutritive Plate

24 h at 37 °C

Confirmation

Performance

UP TO 11 DAYS FOR POSITIVE SAMPLES

The RambaQUICK™ Salmonella method was designed with the aim of simplifying the test procedure while maintaining a high level of sensitivity within a conventional culture methodology.

1. 2 en 1 Enrichment step : The RambaQUICK™ selective broth is a combination of the best functions found in each of both conventional enrichments, the RVs and the MKTTn broths. After the revivification phase in BPW, the *Salmonella* replicates at the exponential growth phase in the optimized RambaQUICK™ Salmonella broth, which offers not only a highly nutrient environment but also inhibits the growth of competitive flora.

2. Fast method :

- > Negative in 2 days,
- > Positive in 3 days.

3. Simple :

- > One selective enrichment,
- > One culture plate.

4. Meets ISO 6579 requirements : by detecting lactose positive *Salmonella* in intense colour on CHROMagar™ Salmonella Plus.

Annex 2. Website information about CHROMagar™ Salmonella Plus

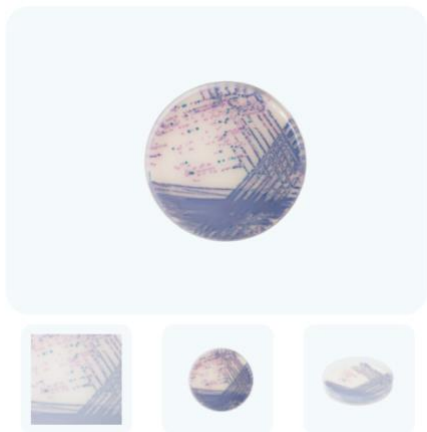
Our Company Products Technical documents **CHROMagar™** The Chromogenic Media Pioneer Find your distributor Contact EN

Clinical Microbiology A complete range of culture media to help in clinical diagnosis

Food Industry A complete range of culture media for the food industry

Water Testing A complete range of culture media for water analysis

Veterinary Microbiology A complete range of culture media for the veterinary sector



CHROMagar™ Salmonella Plus

For detection and isolation of *Salmonella* species including lactose positive *Salmonella*

Order References

Please use these references when contacting your local distributor:

5000 mL Pack SA162
 25 L Pack SA163-25
 CHROMagar™ White Opaque supplement (optional):
 5000 mL Pack SU702
 25 L Pack SU703-25



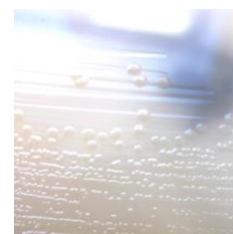
Salmonella, including *S. Typhi*, *S. Paratyphi* and lactose-positive *Salmonella*
 Mauve

Composition

Powder Base	Total 32.8 g/L Agar 15.0 Peptone and Yeast extract 8.0 Salts 8.5 Chromogenic mix 1.3 Storage at 15/30 °C - pH: 7.5 ± 0.2 Shelf Life 3 years
+ Supplement (included in the pack)	1 st : Liquid form 6 mL/L Storage at 15/30 °C Shelf Life 5 years
CHROMagar™ White Opaque supplement	<u>In order to obtain a white opaque background:</u> Powder form : 1.0 g/L Storage at 15/30 °C Shelf Life 3 years
Usual Samples	food, meat, fresh eggs, milk products etc.
Procedure	Inoculation after selective broth enrichment of samples according to ISO 6579. Incubation 18-24 h at 37 °C. Aerobic conditions.
Scientific Publications on this product: available on www.CHROMagar.com Please read carefully the instructions for use (IFU document) available on www.CHROMagar.com	



Coliforms
 Blue



E. coli
 Colorless

Performance

Mainly due to contamination in the food chain and/or during food-production processes, *Salmonella* commonly induces enteric illness whose major symptoms are abdominal cramps, diarrhea, nausea, vomiting.

1. **Meets ISO 6579-1 requirements** by detecting lactose positive *Salmonella* in intense mauve color.

2. **Easy to read by naked eye:** Another feature of this medium resides in its nice colour contrast due to the fact that *E. coli* are colourless. *E. coli*, which are usually present in abundance in samples tested for *Salmonella* and could potentially hide suspect colonies, are no longer a concern.

3. High Sensitivity and Specificity:

Salmonella including lactose positive *Salmonella*: 99 %*

*Specificity and sensibility from scientific study "Evaluation of a new chromogenic medium CHROMagar™ Salmonella Plus for the detection of *Salmonella* species including lactose positive *Salmonella*, *S. typhi* and *S. paratyphi*" de Beaumont C., Breuil J., Dedicova D., Tran Q. 2006.