MICROGEN BIOPRODUCTS

MICROGEN SALMONELLA LATEX AND CHROMAGAR SALMONELLA INTERNAL ASSESSMENT – COMPARISON REFERENCE CULTURES

SCOPE:

The validation study outlined is used to determine the accuracy of the Microgen Salmonella Latex as a rapid confirmation test for cultures isolated on Chromagar Salmonella Plus for the confirmation of pure bacterial cultures belonging to the genus Salmonella***.*** As this study is dealing with pure cultures, the matrix from which the cultures were originally derived will have no bearing on the validation of the test kits.

This validation study will focus on demonstrating the equivalence of the performance of the Microgen Salmonella Latex for the identification of ***Salmonella spp.*** grown on the selective and chromogenic agar plate medium Chromagar Salmonella Plus compared to the performance of the Microgen™ Salmonella Latex for the identification of ***Salmonella spp.*** grown on non selective nutrient agar plate medium.

DEFINITIONS: AS/NZ 4659.3:1999 Section 3

For the purpose of this study, the following definitions apply:

Standard

The reference method against which the trial is being performed is bases on the Australian/ New Zealand Standard AS/NZ 4659.3:1999: Guide to determining the equivalence of food microbiology test methods Part 3: Confirmation Tests. This standard may be downloaded from the Standards Australia Website at www.standards.com.au.

Alternative Method

In the context of this study, the alternative method shall be the Microgen Salmonella Latex for the identification of ***Salmonella spp.*** grown on the selective and chromogenic agar plate medium Chromagar Salmonella Plus

Reference Method

In the context of this study, the reference method shall be Microgen Salmonella Latex for the identification of ***Salmonella spp.*** grown on non selective nutrient agar plate media.

Reference Cultures

In the context of this study, isolates belonging to the genus ***Salmonella spp***. previously identified and confirmed by Microgen GNA ID and in some cases serotyping from the Microgen Culture Collection (MBCC) shall be used.

False Negative AS/NZ 4659.3:1999 Section 3.2

The alternative method yields a result which differs from the reference method which yield the correct result.

False Positive AS/NZ 4659.3:1999 Section 3.3

The alternative method yields a correct result while the reference method yields an incorrect result.

Validate

To determine in accordance with AS/NZ 4659.3:1999 that two methods, (Alternative and Reference) are substantially equivalent when tested against the range of target organisms.

PROCEDURE AS/NZ 4659.3:1999 Section 4

1. **Definition** AS/NZ 4659.3:1999 Section 4.1

   The validation study will demonstrate the equivalence of the Microgen Salmonella Latex as a rapid confirmation test for cultures isolated on Chromagar Salmonella Plus for the identification of Salmonella spp..

   The identification process shall involve:

   - Inoculation of the reference cultures onto both Chromagar Salmonella Plus and Nutrient Agar plates.
   - The performance of the Microgen Salmonella Latex test on isolates grown on both media.
   - Recording of both the strength and time taken to achieve positive agglutination.

2. **Conditions of Trial** AS/NZ 4659.3:1999 Section 4.2

   - The trial shall be undertaken by Karen Ukaegbu
   - The trial laboratory is Microgen Bioproducts
   - The testing is to be performed within a Class 2 laboratory.

3. **Test Organisms** AS/NZ 4659.3:1999 Section 4.3

   In accordance with guidelines provided in AS/NZ 4659.3:1999: Guide to determining the equivalence of food microbiology test methods Part 3: Confirmation Tests, the test panel shall comprise the following:

   Salmonella spp > 40 strains
   Non ***Salmonella spp*** > 30 strains
   (> 30 strains of ***Enterobacteriaceae*** covering closely related and non related strains)

   Closely related strains shall comprise:
   Citrobacter spp. and ***Proteus spp***

A complete list of strains employed in the trial including source and the identification specified identification is provided in Appendix 1.

4. **Preparation of Inoculum** AS/NZ 4659.3:1999 Section 4.4

   All test organisms are currently stored in the Microgen Bioproducts Culture Collection (MBCC) laboratories culture collection STORED AT -80°C.

   Prior to use, strains shall be sub cultured onto CLED Agar Plates (Oxoid) and incubated at 37°C for a minimum of 18 hours but not exceeding 24 hours. All sub cultures will be checked for viability, colonial morphology (lactose/ non lactose fermentation) and purity.

5. **Testing** AS/NZ 4659.3:1999 Section 4.5

   Each test organism shall be sub cultured from the CLED agar plates onto both Chromagar Salmonella Plus and the nutrient agar plate medium. These plates shall be incubated at 37°C for a minimum of 18 hours but not exceeding 24 hours.

   A single colony from both the Chromagar Salmonella Plus and the nutrient agar plate medium shall be evaluated simultaneously using the Microgen Salmonella Latex the same operator using the method described in the Microgen Salmonella Latex Instructions for use.

   The time taken to achieve either:

   1. 3+ Agglutination (full background clearing of the reaction mixture, or
   2. The strength of the agglutination reactions after 2 minutes (if weaker than 3+) shall be recorded.

   The final agglutination results obtained shall be recorded in Appendix 1

6. **Results** AS/NZ 4659.3:1999 Section 4.6

   The final Results shall be correlated in accordance with AS/NZ 4659.3:1999: Guide to determining the equivalence of food microbiology test methods Part 3: Confirmation Tests.

   The following statistics shall be calculated:

   - Sensitivity
   - Specificity
   - False Positive
   - False Negative

   Using the following algorithms:

Nutrient Agar Method	**Chromagar Method**	
	Positive	Negative
Positive	A	B
Negative	C	D

Specificity = A/(A + B) x 100 Sensitivity = D/(C + D) x 100

False Positive = C/(A + B) False Negative = B/(C + D)

Acceptance Criteria:

The Microgen Salmonella Latex results from the Chromagar Salmonella Plus shall be deemed to be equivalent if B=0 (NO FALSE NEGATIVE RESULTS WITH THE ALTERNATIVE SYSTEM).

See Appendix 1 for Final Results and Analysis

APPENDIX 1

Reference Culture Final Results

RESULTS:

	MBCC No.	Salmonella Organisms	1 colony Salmonella latex (M42a) lot 106 Agglutination results at 2 mins			Sweep of colonies Salmonella latex (M42a) lot 106 Agglutination results at 2 mins			Comments
			CLED Agar	Nutrient Agar	Salmonella Chromagar	CLED Agar	Nutrient Agar	Salmonella Chromagar	
1	115	S. albany	3+ 50s	-	-		3+ 45s	3+	
2	116	S. alandale	-	-	-				
3	117	S. anatum	3+ 20s	3+ 45s	3+ 25s				
4	295	S. anatum	3+ 15s	3+ 25s	3+ 10s				
5	118	S. assinie	3+ 20s	3+ 50s	-			3+ 20s	
6	119	S. bergen	3+ 15s	-	-		-	-	
7	120	S. berkeley	-	-	-				
8	331	S. berkeley	-	-	-		-	- cl	
9	121	S. bolombo	3+ 15s	3+ 90s	3+ 15s				
10	122	S. california	3+ 15s	3+ 60s	3+ 15s				
11	123	S. coeln	3+ 90s	3+ 60s	3+ 20s				
12	124	S. cubana	-	-	-				
13	301	S. cubana	3+ 60s	3+ 90s	-	3+ 50s	3+ 25s	3+ 90s	
14	345	S. cubana	-	3+	3+ 65s	3+ 25s			
15	125	S. derby	3+ 70s	-	-		+	2/+	
16	281	S. derby	3+ 15s		3+ 10s				
17	126	S. deversoir	-	-	-				
18	127	S. dublin	-	-	-				Straw coloured colonies
19	128	S. dublin	-	-	-				Straw coloured colonies
20	129	S. dublin	-	- gr	-				Straw coloured colonies
21	130	S. dublin	-	-	-				Straw coloured colonies
22	131	S. dublin	-	-	-				Straw coloured colonies
23	132	S. dublin	-	-	-				Straw coloured colonies
24	133	S. dublin	3+ 20s	3+ 100s	3+ 50s				
25	280	S. dublin	3+ 10s	3+ 15s	3+ 15s				
26	134	S. enteritidis	-	-	-				
27	135	S. enteritidis	-	-	- cl				
28	136	S. enteritidis	-	-	-				
29	137	S. enteritidis	-	-	-				
30	192	S. enteritidis	+	+/-	3+				

	MBCC No.	Salmonella Organisms	1 colony Salmonella latex (M42a) lot 106 Agglutination results at 2 mins			Sweep of colonies Salmonella latex (M42a) lot 106 Agglutination results at 2 mins			Comments
			CLED Agar	Nutrient Agar	Salmonella Chromagar	CLED Agar	Nutrient Agar	Salmonella Chromagar	
31	138	S. gaminara	-	-	-				
32	139	S. glostrup	3+ 90s	- gr	3+ 35s		3+ 20s	3+ 20s	
33	140	S. hadar	-	- gr	-				
34	141	S. hadar	3+ 40s	3+ 60s	3+ 35s				
35	142	S. heidelberg	3+ 30s	3+ 70s	3+ 25s				
36	143	S. heidelberg	3+ 55s	3+ 35s	3+ 90s				
37	144	S. hvittingfoss	-	-	-				
38	145	S. indiana	-	-	-				Straw coloured colonies
39	146	S. infantis	3+ 10s	3+ 45s	3+ 25s				
40	147	S. karamoja	-	-	-				Blue colonies
41	148	S. kingston	3+ 10s	3+ 25s	3+ 60s		3+ 10s	3+ 10s	
42	149	S. kubacha	3+ 10s	3+ 40s	3+ 90s		3+ 10s	3+ 10s	
43	150	S. lille/rumforal	3+ 15s	+	+		3+ 10s	3+ 10s	
44	151	S. miami	3+ 20s	3+ 25s	3+ 25s				
45	152	S. ndolo	3+ 15s	3+ 15s	3+ 15s				
46	153	S. newbrunswick	+/-	-	-	3+ 65s	3+ 65s	3+ 65s	Purple colonies
47	154	S. ohio	3+ 90s	2/3+	2/3+				
48	155	S. panama	-	-	-	3+ 80s	3+ 100s	2/3+	Purple colonies
49	156	S. rutgers	3+ 45s	2/3+	3+ 45s				
50	157	S. senftenburg	3+ 30s	3+ 25s	3+ 70s				
51	158	S. senftenburg	3+ 15s	3+ 15s	3+ 70s				
52	282	S. senftenburg	3+ 15s	3+ 15s	3+ 25s				
53	159	S. tennessee	3+ 20s	3+ 30s	3+ 30s				
54	160	S. thomson	2+	2+	2+				
55	165	S. typhimuruim	3+ 20s	3+	3+ 30s				
56	172	S typhimuruim	3+ 15s	3+ 15s	3+ 15s				
57	173	S. typhimuruim	3+ 25s	3+ 15s	3+ 60s				
58	174	S. typhimuruim	3+ 50s	3+ 15s	3+ 60s				

	MBCC No.	Salmonella Organisms	1 colony Salmonella latex (M42a) lot 106 Agglutination results at 2 mins			Sweep of colonies Salmonella latex (M42a) lot 106 Agglutination results at 2 mins			Comments
			CLED Agar	Nutrient Agar	Salmonella Chromagar	CLED Agar	Nutrient Agar	Salmonella Chromagar	
59	175	S. typhimuruim	3+ 30s	3+ 20s	3+ 35s				
60	191	S. typhimurium	3+ 20s	3+ 15s	3+ 35s				
61	215	S. typhimurium	3+ 10s	3+ 20s	3+ 20s				
62	304	S. typhimurium	3+ 15s	3+ 70s	3+ 100s				Purple
63	171	S. tranoroa	- (cl)	-	-	- (cl)	- (cl)	- (cl)	Pale purple/straw colonies
64	176	S. virchow	3+ 15s	3+ 20s	3+ 20s				
65	182	Salmonella spp	3+ 20s	3+ 20s	3+ 70s				
66	183	Salmonella spp	3+ 10s	3+ 15s	3+ 25s				
67	184	Salmonella spp	-	-	-	-	-	-	Green colonies
68	185	Salmonella spp	-	-	-	- (v.cl)	- (v.cl)	- (v.cl)	Purple colonies
69	186	Salmonella spp	-	-	-	- (cl)	- (cl)	- (cl)	Purple colonies
70	187	Salmonella spp	3+ 20s	3+ 25s	3+ 25s				
71	188	Salmonella spp	3+ 30s	3+ 25s	3+ 25s				
72	216	S. poona	3+ 50s	3+ 25s	3+ 45s				
73	292	S. poona	3+ 25s	3+ 40s	3+ 80s				
74	274	S. bispeberg	3+ 10s	3+ 10s	3+ 10s				
75	329	S. bispeberg	3+ 25s	3+ 30s	3+ 30s		3+ 10s	3+ 10s	
76	275	S. java	3+ 10s	3+ 10s	3+ 10s				
77	297	S. jaja? java	3+ 50s	3+ 50s	+			3+ 20s	
78	330	S. java	3+ 40s	+/-	3+ 80s				
79	298	S. ridge	3+ 85s	2/+	2/+				
80	299	S. glostrup	3+ 25s	3+ 20s	3+ 20s				
81	300	S. blukwa	3+	3+	-			3+	
82	344	S. blukwa	-	+	-	3+ 85s		3+ 40s	
83	302	S. lille	3+ 20s	+/-	+	3+ 15s	3+ 15s	3+ 25s	Purple colonies
84	303	S. sandiego	3+ 25s	2/3+	3+ 25s				

	MBCC No.	Salmonella Organisms	1 colony			Sweep of colonies			
			Salmonella latex (M42a) lot 106 Agglutination results at 2 mins			Salmonella latex (M42a) lot 106 Agglutination results at 2 mins			
			CLED Agar	Nutrient Agar	Salmonella Chromagar	CLED Agar	Nutrient Agar	Salmonella Chromagar	
85	332	S. birkenhead	3+ 70s	3+ 65s	3+ 40s				
86	277	S. virginia	3+ 25s	3+ 25s	3+ 25s				
87	333	S. virginia	3+ 100s	-	-		3+ 15s	3+ 15s	
88	293	S. saint-paul	3+ 25s	3+ 30s	3+ 30s				
89	334	S. saint-paul	3+ 40s	3+ 70s	3+			3+ 50s	
90	294	S. choleraesuis	3+ 25s	3+ 30s	3+ 10s				(2 colonies used)
91	335	S. rubislaw	2/+	-	3+ 70s				
92	283	S. rostock	-	-	2+				Straw coloured colonies
93	336	S. rostock	-	-	-	3+ 65s	3+ 75s	3+ 75s	
94	284	S moscow	3+ 50s	3+	+				
95	337	S moscow	-	-	-	-	-	-	
96	285	S. montevideo	3+ 20s	3+ 15s	3+ 35s				(2 colonies used)
97	338	S. montevideo	3+ 40s	3+ 85s	3+ 45s			3+ 10s	
98	286	S. oranienberg	3+ 20s	3+ 15s	3+ 90s				
99	339	S. oranienberg	3+ 30s	3+ 50s	3+ 15s				
100	288	S. london	3+ 20s	3+ 20s	3+ 35s				(2 colonies used)
101	340	S. london	3+ 20s	3+ 40s	3+ 45s				
102	289	S. meleagridis	3+ 20s	3+ 20s	3+ 35s				(2 colonies used)
103	341	S. meleagridis	3+ 35s	3+ 40s	3+ 50s				
104	291	S. telaviv	2/3+	3+	3+ 100s				
105	342	S. telaviv	-	3+ 100s	-	3+ 60s	3+ 50s	3+ 50s	
106	296	S. give	3+ 30s	3+ 25ss	3+ 50s				
107	343	S. give	3+ 65s	3+ 45s	3+ 45s				
108	346	S. sandiego	3+ 20s	3+ 20s	3+ 45s				
109	347	S. banalia	3+ 95s	3+ 40s	3+ 75s				
110	348	S. waycross	-	+/-	-	+ (cl)	+ (cl)	-	
111	356	S. Nottingham	+/- (cl)	-	- (cl)	- (cl)	- (cl)	- (cl)	Purple colonies

NON-SALMONELLA STRAINS

	MBCC No.	Salmonella Organisms	1 colony Salmonella latex (M42a) lot 110 Agglutination results at 2 mins			Comments
			CLED Agar	Nutrient Agar	Salmonella Chromagar	
1	617	Morganella spp	-	-	-	Signs of contam.
2	76	Proteus mirablis	-	- (cl)	-	
3	77	Proteus mirablis	-	-	-	
4	78	Proteus mirablis	-	-	-	
5	166	Proteus mirablis	-	-	-	
7	607	Proteus spp	-	-	-	
8	608	Proteus spp	-	-	-	
9	609	Proteus spp	-	-	-	
10	610	Proteus spp	-	-	-	
11	611	Proteus spp	-	-	-	
12	612	Proteus spp	-	-	-	
13	613	Proteus spp	- (cl)	- (cl)	-	Dark blue colonies
14	614	Proteus spp	- (cl)	- (cl)	-	Dark blue colonies
15	615	Proteus spp	-	-	-	
16	616	Proteus spp	-	-	-	
17	74	Proteus vulgaris	**NOT IN COLLECTION**			
18	75	Proteus vulgaris	-	-	- (cl)	Green colonies
19	213	Proteus vulgaris				
20	627	Providencia spp	-	-	-	Straw coloured
21	168	Providencia stuartii				
22	65	Citrobacter diversus				
23	60	Citrobacter freundii	- (cl)	- (cl)	- (cl)	Green colonies
24	61	Citrobacter freundii	- (cl)	-	-	
25	62	Citrobacter freundii	**NOT IN COLLECTION**			
26	63	Citrobacter freundii	- (cl)	-	-	Green colonies
27	64	Citrobacter freundii	- (cl)	-	- (cl)	Green colonies
28	84	Citrobacter freundii	-	-	- (cl)	Pale green colonies
29	85	Citrobacter freundii	- (cl)	- (cl)	- (cl)	Green colonies
30	86	Citrobacter freundii	- (tr)	-	- (tr)	
34	623	Citrobacter spp	- (cl)	- (cl)	-	Green colonies
35	624	Citrobacter spp	- (cl)	-	-	Green colonies

ANALYSIS OF RESULTS:

Nutrient Agar Method	Chromagar Method	
	Positive	Negative
Positive	82	0
Negative	0	11

Specificity: 100%
Sensitivity: 100%
False Positive: 0%
False Negative: 0%

Note: Due to factors discussed in the conclusion, the Salmonella Latex results are the combined results of both the testing of single colonies and the testing of multiple colonies (sweep).

On the basis that the Microgen Salmonella Latex results from the Chromagar Salmonella Plus produced no false positive results, the Microgen Salmonella Latex testing of colonies from the Chromagar Salmonella Plus is deemed to be equivalent to the results from the non selective Nutrient Agar Plate Media.

COMMENTS:

The general pattern seen throughout this exercise is that a colony from a chromagar plate can produce agglutination reactions comparable to a colony picked from a nutrient agar. However, occasions did arise where stronger/or faster reactions were observed from a colony on a chromagar than on a nutrient agar and vice versa. This most probably due to variations in the size of colonies, hence the quantity of antigen employed in the test. Colony size varied between a pin prick to something significantly larger – generally the bigger the colony size the faster the reactions and the smaller the colony size the slower the positive reactions. It was because of this variation in colony size that in some cases it was decided to test a sweep of colonies to compare difference in agglutination speed and reaction. In general, the reactions were a sweep of colonies was used was much faster and stronger than a reaction from a single colony, this being due to the higher concentration of organism/ antigen used in the test.

It should also be noted that the Microgen Salmonella Latex test is based on the detection of flagella antigens which are associated with the motility of the Salmonella organisms. All of the cultures tested were taken from the Microgen Culture Collection. Long term storage of Salmonella cultures is well known to reduce or eliminate the motility of the organisms. Motility can be regained readily by passing cultures multiple times, or passing through motility media. It may be that the cultures that tested poorly with a single colony had not regained optimum motility and flagella antigen expression. By testing multiple colonies i.e. sweep, sufficient antigen was applied to the latex agglutination test.

Philip Mugg
12/04/16

Karen Ukaegbu
12/04/16

APPENDIX 2

Manufacturer’s instructions for Identification Kits Under Evaluation

MICROGEN BIOPRODUCTS

F42 MICROGEN® SALMONELLA

INTENDED USE

Microgen® Salmonella is a latex slide agglutination test for the confirmatory identification of presumptive Salmonella colonies from selective agar plates. The kit is **not** intended for clinical use.

PRINCIPLE OF THE TEST

Latex particles are coated with polyvalent antisera raised against a wide range of Salmonella antigens. When mixed with a suspension of Salmonella organisms, the latex particles rapidly agglutinate to form visible clumps. Microgen® Salmonella detects >99% of motile Salmonella species and early investigations have indicated that specific non-motile species may also be detected.

KIT PRESENTATION

F42a Salmonella Latex Reagent: Latex particles coated with rabbit antiserum against Salmonella antigens. Preserved with 0.099% sodium azide. (**Blue** cap) 2.5mL

F42b Positive Control: Inactivated preparation of Salmonella antigens preserved with 0.099% sodium azide. (**Black** cap) 0.5mL

F40 0.85% Isotonic Saline: Preserved with 0.099% sodium azide. (**White** cap) 5.0mL

Instructions for Use
Disposable agglutination slides
Disposable mixing sticks

Additional Requirements:

- Bacteriological loops
- Pasteur pipettes

WARNINGS AND PRECAUTIONS

Safety:

1. The reagents supplied in this kit are for *in vitro* diagnostic use only
2. Sodium azide, which is used as a preservative in the kit reagents can react with lead or copper plumbing to form potentially explosive metal azides. Dispose by flushing with a large volume of water to prevent azide build-up.
3. Appropriate precautions should be taken when handling or disposing of potential pathogens. Decontamination of infectious material can be achieved with sodium hypochlorite at a final concentration of 3% for 30minutes. Liquid waste containing acid must be neutralised before treatment.
4. The positive control has been inactivated during the manufacturing process. However, it should be handled as though potentially infectious.

Procedural:

1. Microgen® Salmonella should be used according to the kit instructions.
2. Allow all reagents to reach room temperature before use.
3. Do not dilute any of the kit reagents
4. Do not intermix reagents from different batches of kits.
5. Do not freeze any of the kit reagents
6. Do not allow the latex reagent dropper to touch the positive control or bacterial samples.
7. Be careful only to record agglutination. Reactions that are "curdy" or "stringy" may not be true agglutination.
8. Ensure the slide is clean and dry prior to use.

STORAGE AND SHELF LIFE

Microgen® Salmonella should be stored at 2-8°C when not in use. The kit should not be used after the expiry date printed on the carton label.

SPECIMENS

Colonies grown on selective agar plates can be tested with Microgen® Salmonella.

PROCEDURE

Quality Control:

The following controls should be performed each time the kit is used.

1. Reagent Control: Add one drop of Microgen® Salmonella latex (F42a) to one drop of F40 saline solution in the same circle on a slide. Mix and spread the liquid over the entire area of the circle with a mixing stick. Rock the slide gently for 2 minutes and observe for agglutination. If any agglutination is seen, either the latex or the saline is contaminated and should be discarded.
2. Positive Control: Add one drop of positive control (F42b) to one circle on the test slide. Add one drop of Microgen® Salmonella latex to the same circle and mix. **Do not allow the dropper to touch the positive control.** Rock the slide gently. Within 2 minutes, agglutination, indicating a positive result, should be visible. If no agglutination is seen, a fresh kit should be used.

Test Procedure:

1. Dispense 1 drop of F40 isotonic saline into a circle of a Microgen® agglutination slide.
2. Using an inoculating loop, remove a colony from the selective agar plate and emulsify the colony in the drop of saline to produce a heavy smooth suspension. Suspensions should only be made from colonies with morphologies resembling *Salmonella spp.*
3. Rock the slide gently for up to 2 minutes and observe for auto-agglutination or clumping. If the suspension remains smooth, proceed to Step 4 (see Limitations of Use Note 1).
4. Mix the Microgen® Salmonella latex by gently inverting and add one drop next to the bacterial suspension. **Do not allow the dropper to touch the suspension.**
5. Mix the latex reagent and the bacterial suspension with a clean mixing stick and rock the slide gently two or three times. Excessive rocking of the slide is not necessary. Examine for agglutination within a maximum of 2 minutes.
6. After reading, discard the used mixing sticks and slides into suitable disinfectant.

INTERPRETATION

Agglutination within 2 minutes is a positive result and indicates the presence of Salmonella in the sample. Absence of agglutination indicates that Salmonella is not present in the test culture.

LIMITATIONS OF USE

1. Results should be interpreted in the context of all available clinical and laboratory information.
2. Rough strains of Salmonella are known to cause non-specific auto-agglutination in saline alone and therefore cannot be tested with Microgen® Salmonella.
3. Some non-motile strains may not be detected by Microgen® Salmonella.
4. Some oxidase-positive organisms may give false positive reactions.
5. Old stock cultures of Enterobacteriaceae on nutrient agar slopes may cause non-specific agglutination whereas old stocks of Salmonella may give false negative results. Fresh sub-cultures should be prepared for testing.
6. Identification with Microgen® Salmonella is presumptive and all positive results should be confirmed by further identification tests and serotyping of pure cultures.

PERFORMANCE CHARACTERISTICS

Microgen® Salmonella has been evaluated in comparison with a well-established commercially available latex agglutination test for Salmonella. 126 isolates of *Salmonella spp.* and a range of 58 potentially cross-reacting bacteria were tested in both products.

		Microgen® Salmonella		
		+ve	-ve	Total
Commercial Latex Test	+ve	135**	1*	136
	-ve	0	48***	48
	Total	135	49	184

Sensitivity: 135/136 = 99.3%
Specificity: 48/48 = 100%
Concordance: 183/184 = 99.5%

*1 sample was negative in Microgen® Salmonella but equivocal in the commercial test. This sample was subsequently identified as *Salmonella bergen.*

** Of the 135 isolates in this group, 11 were cross reactants in both tests. These were isolates of *C. diversus* (1), *A. baimannii* (2), *P. stuartii* (1), *B. cereus (2), S.aureus* (4), *Strep spp* (1)
However all of the above either did not grow or showed very atypical morphologies, when cultured on Salmonella-selective media. In the case of B. cereus, agglutination was atypical (stringy)

*** 1 *S. dublin* was repeatedly negative in both tests.

REPRODUCIBILITY

Intra-batch reproducibility was evaluated by testing sensitivity and specificity of 1 batch of product against serial dilutions of reference and kit control antigens, and a panel of 34 bacterial samples. Different operators carried out tests on 3 separate occasions. End-point titres obtained with reference/control antigens and qualitative results with the panel were identical in the three assays.

Inter-batch reproducibility was examined by testing sensitivity and specificity of 3 batches of product against serial dilutions of reference and kit control antigens, and a panel of 34 bacterial samples. Between the 3 batches, variation in end-point titres was minimal (1 doubling dilution) and qualitative results with the panel correlated 100%.

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WF5049/2006/06

Manufacturers instructions for CHROMagar Salmonella Plus Under Evaluation

Instructions For Use

CHROMagar™ Salmonella Plus

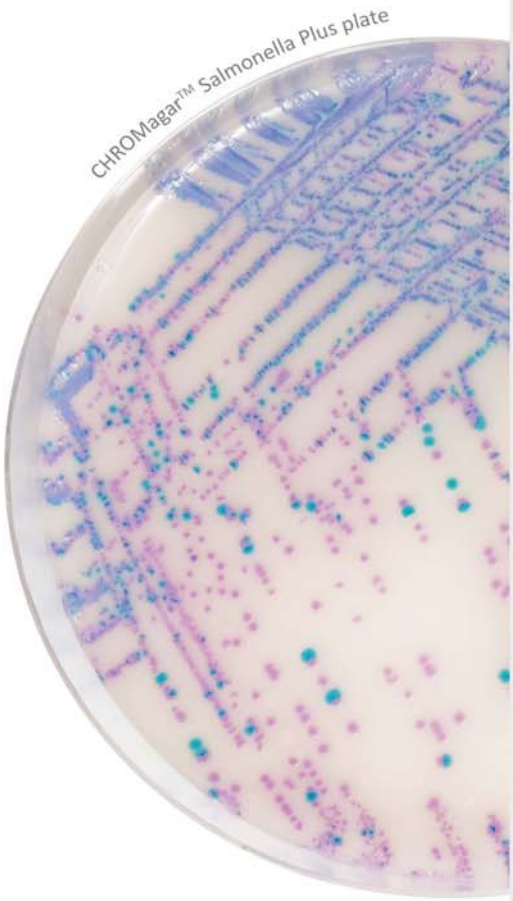
Instructions For Use

Available in several languages

NT-EXT-024

Version **10**

ENGLISH	English Version
FRANCAIS	Version Française
ESPAÑOL	Version Español
DEUTSCH	Deutsch Version
日本	日本版



CHROMagar
The Chromogenic Media Pioneer

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CHROMagar™ Salmonella Plus

MEDIUM PURPOSE

Chromogenic medium for the isolation of Salmonella species including *S.*Typhi and lactose positive *Salmonella*.
This medium meets the ISO 6579 : 2002 ISO norm.
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, and vomiting.
Second most important foodborne pathogen, its detection remains a big concern in the medical field, and a big challenge in the food industry including the lactose plus strains largely encountered in the poultry.

COMPOSITION

The product is composed of a powder base (B) , 1 supplement (S) and 1 optional supplement.

Product =	Base (B)	Supplement (S)	OPTIONAL USE White Opaque Supl.
Total g/L	32.8 g/L	6.0 ml/L	1.0 g/L
Composition g/L	Agar 15.0 Peptone and yeast extract 8.0 Salts 8.5 Chromogenic mix 1.3	Growth mix 6.0	
Aspect	Powder Form	Liquid Form	Powder Form
STORAGE	**15-30°C**	**15-30°C**	**15-30°C**
FINAL MEDIA pH		7.5 +/- 0.2	

When and why should I use the CHROMagar White Opaque Supplement?

The white opaque has to be incorporated in the preparation of the medium for an opaque background.

PREPARATION (Calculation for 1L)

Step 1 — Preparation of base (B) + (S)

- Disperse slowly 32.8g of powder base in 1L of purified water.
- Stir until agar is well thickened.
- Add 6ml of the LIQUID content of the supplement.
  Warning 1: To obtain a white opaque background, look at Step 2.
- Swirl for mixing.

OPTIONAL

In order to obtain a white opaque background:

Step 2 — Preparation of white opaque background

- Put 1g of the CHROMagar White Opaque supplement reference SU702 in 10 ml purified water.
- Vortex well for homogenization. Appearance: milky suspension.
- Add the 10ml to the rehydrated base before the heating step.

Step 3 — Heating

- Heat and bring to boil (100°C) while swirling or stirring regularly.

DO NOT HEAT TO MORE THAN 100°C. DO NOT AUTOCLAVE AT 121°C.
Warning 2: If using an autoclave, do so without pressure.
Advice 1: For the 100°C heating step, mixture may also be brought to a boil in a microwave oven: after initial boiling, remove from oven, stir gently, then return to oven for short repeated bursts of heating until complete fusion of the agar grains has taken place (large bubbles replacing foam).

- Cool in a water bath to 45-50°C.

Advice 2: in case of product samples containing a high load of *Pseudomonas* and/or *Aeromonas*, Cefsulodin can be added at 5 mg/L.

Step 4 — Pouring

- Swirl or stir gently to homogenize.
- Pour into sterile Petri dishes.
- Let it solidify and dry.

Storage

- Store in the dark before use.
- Prepared media plates can be kept for one day at room temperature.
- Plates can be stored for up to one month under refrigeration (2/8°C) if properly prepared and protected from light and dehydration.

CHROMagar™ Salmonella Plus

Instructions For Use

ENGLISH

Instructions For Use

INOCULATION

Related samples can be processed by direct streaking on the plate, as well as prior appropriate enrichment step *(RambaQUICK Salmonella enrichment broth is available : reference SQ001).*

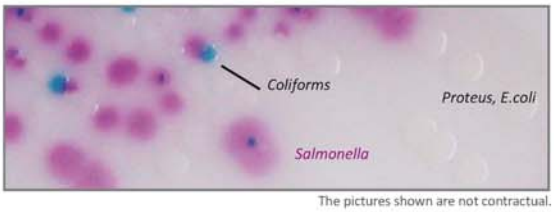
- If the agar plate has been refrigerated, allow to warm to room temperature before inoculation.
- Streak sample onto plate.
- Incubate in aerobic conditions at 37°C for 18-24 hours.

Typical Samples
e.g. food, meat, fresh eggs, dairy milk products as well as medical samples: blood, stools etc *** Possible enrichment step Direct streaking or spreading technique

INTERPRETATION

Microorganism	Typical colony appearance
Salmonella (including *S.*Typhi, *S.*paratyphi A and lactose positive *Salmonella*)	→ mauve
E.coli	→ colourless
Coliforms	→ blue
Proteus	→ colourless or inhibited

Typical colony appearance



The pictures shown are not contractual.

PERFORMANCE & LIMITATIONS

- Final identification must be done by biochemistry and serology, and can be performed directly from the plates on suspected colonies.
- This medium has a very high sensitivity but some *Salmonella Dublin* may appear colourless, nevertheless *Salmonella Dublin* is a rarely encountered serovar.
- Some *E.coli* strains may develop a very slight mauve colouration.
- Some *Pseudomonas* may have similar mauve colony aspect and can be eliminated by an Oxydase test.

QUALITY CONTROL

Please perform Quality Control according to the use of the medium and the local QC regulations and norms.
Good preparation of the medium can be tested, isolating the ATCC strains below:

Microorganism	Typical colony appearance
S.abaetuba ATCC® 35640	→ mauve
S.typhimurium ATCC® 13311	→ mauve
S.enteritidis ATCC® 13076	→ mauve
S.arizonae CIP 8230	→ mauve
E.coli ATCC® 25922	→ colourless
C.freundii ATCC® 8090	→ blue
S.aureus ATCC® 25923	→ inhibited
P.aeruginosa ATCC® 9027	→ inhibited

WARNINGS

- Do not use plates if they show any evidence of contamination or any sign of deterioration.
- Do not use the product beyond its expiry date or if product shows any evidence of contamination or any sign of deterioration.
- For *in vitro* diagnostic use. This laboratory product should be used only by trained personnel in compliance with good laboratory practices.
- Any change or modification in the procedure may affect the results.
- Any change or modification of the required storage temperature may affect the performance of the product.
- Unappropriate storage may affect the shelf life of the product.
- Recap the bottles/vials tightly after each preparation and keep them in a low humidity environment, protected from moisture and light.
- For a good microbial detection: collection and transport of specimen should be well handled and adapted to the particular specimen according to good laboratory practices.

REFERENCES

Please refer to our website page «Publications» for scientific publications about this particular product.
Web link: http://www.chromagar.com/publication.php

DISPOSAL OF WASTE

After use, all plates and any other contaminated materials must be sterilized or disposed of by propriate internal procedures and in accordance with local legislations. Plates can be destroyed by autoclaving at 121°C for at least 20 minutes.

IFU/LABEL INDEX

- Quantity of powder sufficient for X liters of media
- Expiry date
- Required storage temperature
- Store away from humidity

Pack Size			Ordering References				
5000 ml	=	250 Tests of 20ml	SA162	=	SA162(B)	+	SA162(S)
5000 ml	=		SU702	=	White Opaque Suppl.		
25 L	=	1250 Tests of 20ml	SA163-25	=	SA163-25(B)	+	SA163-25(S)
25 L	=		SU703-25	=	White Opaque Suppl.		

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