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# Enumeration medium study for enterobacteria by enumeration of colonies in food products

## January 2018 report

## ■ I. MOTIVATIONS

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CHROMagar requested ACTALIA to study an Enterobacteria enumeration medium for the analysis of food products. This study aims:

- firstly, to test the routine use of the CHROMagar Enterobacteria medium in parallel with the VRBG medium on 50 food samples,
- secondly, to extend the study to a larger number of samples, to test the medium on pure strains and to test the single layer technique in place of the double layer technique.

**The present proposal concerns the analysis of 50 duplicated positive samples using the AFNOR method and the CHROMagar Enterobacteria medium.**

## ■ II. DESCRIPTION OF FOODSTUFFS

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The proposed technical approach is a study of the enumeration of Enterobacteria in naturally contaminated food, using the CHROMagar Enterobacteria medium in comparison with the VRBG medium.

The foodstuffs analysed correspond to products for which a criterion exists for enterobacteria, and products known to be contaminated by this family naturally.

Food of animal origin, seafood and plant products have been studied, as Enterobacteria encompass a great diversity of species, and the species encountered are very different from one product to another. Environmental species (generally psychrotrophic) are distinguished from species found in water and soil etc., belonging to the genera *Rahnella*, *Erwinia*, *Serratia* and others) and species present in the gastrointestinal tract of humans and animals (belonging to genera *Escherichia*, *Enterobacter*, *Citrobacter*, etc.).

Processed foods with hygiene criteria related to Enterobacteria according to European Regulation (EC) 2073/2005 are described in Table 1.

**Table 1 Hygiene criteria relating to *Enterobacteriaceae* for processed products<sup>(1)</sup> according to Regulation (EC) 2073/2005**

	Microbiological limits	
	m	M
Pasteurised milk	1/ml	5/ml
Powdered milk	10/g	
Ice cream	10/g	100/g
Egg products	10/g	100/g

<sup>(1)</sup> There are also criteria for sample from the surface of animal carcasses

At the national level, the Federation of Trade and Industry (*Fédération du commerce et de l'industrie* - FCD) sets criteria for enterobacteria in a wide variety of products:

- meat products: microbiological limits vary according to the type of products (50/g for ham for example, 1000/g for cured sausage, 10,000/g for raw products such as sausages),
- milk desserts, margarine and butter spreads spread (10/g)
- raw meat (1,000 to 10,000/g), offal (50,000 to 500,000/g)
- catering products (fresh pasta 100/g, sandwiches 1,000/g).

Certain products, known to be often highly contaminated with Enterobacteriaceae and for which their presence can not be attributed to a lack of control of the processes involved, are not subject to criteria for enterobacteria. These include, for example, cheeses, raw poultry, processed vegetables

and seafood. For these products, other criteria are taken into account such as coliforms or *E. coli*, which belong to the enterobacteria, but better reflect faecal contamination. However, for these products, enterobacterial enumerations may be carried out within the framework of studies of microbial ecosystems, or during the validation of technological processes.

The study initially focused on 50 samples with enterobacterial contamination, the analysed products having been chosen to cover a range of scenarios with food of different origins and having reasonably predictable higher/lower contamination levels. The list of food products analysed is provided in Table 2. This involves either double samples analysed by the laboratory or products bought on the market.

**Table 2 List of food products analysed**

Categories of products	Products	Description	FCD criteria
Milk products (9)	Cheese (4)	Pasteurised camembert	No criteria
		Crottin de Chavignol cheese	
		Soft cheese, brand Pont l'Eveque	
		Soft cheese, brand Livarot	
	Milk based dessert (3)	Cream cheese (faisselle type)	10/g
		Meringue and custard dessert	
		Egg custard	
Milk (2)	Cream <sup>(1)</sup>	No criteria 1 to 5/ml	
	Curdled milk <sup>(1)</sup>		
Meat products (14)	Meat (6)	Stuffed veal parcels <sup>(1)</sup> 175	1,000 to 10,000/g
		Stuffed veal parcel <sup>(1)</sup>	
		Organic stuffed veal parcels <sup>(1)</sup> 310	
		Minced beef	
		Minced beef	
		Minced beef, butcher	
	Offal (3)	Heart (beef)	50,000 to 500,000/g
		Liver (cattle)	
		Kidney (pork)	
	Poultry (5)	Poultry frankfurter	No criteria
		Turkey breast	
		Chicken leg with skin	
		Sliced chicken fillet	
		Turkey breast	
Cured meat products (13)	Ham (4)	Sliced ham	50/g
		Sliced ham	
		Sliced braised ham	
		Ham with rind	
	Bacon morcels (2)	Thin bacon morcels	10,000/g
		Bacon morcels	
	Chipolatas, sausages (7)	Herb and onion sausage	10,000/g
		Chipolatas	
		Toulouse sausage	
		Cured sausage	
Sausage			
Tunisian spiced sausage			
Chipolatas			
Seafood products (11)	Transformed seafood products (6)	Scottish smoked salmon	No criteria
		Smoked salmon morcels	
		Smoked trout	
		Norwegian smoked salmon	
		Danish smoked salmon	

		Cooked shrimps	
	Raw fish fillets under modified atmosphere (5)	Salmon steak with skin	No criteria
		Pollock	
		cod	
		Pouting	
		Whiting fillet	
Catering products (12)	Pies, quiches, pizza (2)	Pizza with goat cheese	100/g
		Pizza 3 cheeses	
	Salad with dressing (5)	Herring and potato in oil	1000/g (at 37°C) Criterion not retained in case of raw vegetables or cheese
		Ham and egg salad	
		Savoury semolina	
		Greek mushroom dish	
		Apple, cheese and tripe sausage salad	
	Sandwiches (5)	Goat cheese wrap	
		Chicken caesar wrap	
		Ham sandwich with salad and mozzarella	
Egg and ham sandwich			
Roast chicken sandwich			
Pastries (4)	Uncooked (2)	Almond and cream pastry dessert	No criteria
		Chocolate cream pastry dessert	
	Cooked (2)	Strawberry tart	
		Raspberry tart	
Vegetable products (6)	Salad, low quality (6)	Batavia lettuce	No criteria
		Red cabbage	
		Grated carrots	
		Curly Lettuce	
		Variegated lettuce	
		Lettuce	

(1) double sample frozen

### ■ III. PROTOCOL

Regulation 2073/2005 specifies that ISO 21528 2 (colony enumeration technique) should be used for counts of *Enterobacteriaceae*. The FCD recommends the routine norm Afnor NF V 08-054 (Enumeration of Enteric *Enterobacteriaceae* - no confirmation).

Both standards allow for enumeration at 30°C or 37°C. The temperature of 37°C is proposed when enterobacteria are considered as an indicator of hygiene, while the temperature of 30°C can be used as part of a technological process for foods that may contain psychrotrophic enterobacteria.

In consultation with CHROMagar, enumerations were carried out **according to NF V 08-054** on CHROMagar and VRBG media using pouring method **with a double layer** and **incubated at 37°C**.

### III.1. Preparation of media

The references of the media used are provided below.

Medium	reference	Batch/expiry
CHROMagar Enterobacteria base <sup>(1)</sup>	X214B-5	Batch P001143 Expiry date 30/08//2019
CHROMagar Enterobacteria S1	X214S1	Batch 93129 Expiry date 30/08/2019
CHROMagar Enterobacteria S2	X214S2	Batch 93129 Expiry date 30/08/2021
VRBG (violet red bile agar)	OXOID CM 0485	Batch 1486782 Expiry date 30/04/2019

<sup>(1)</sup> technical sheet attached.

Both media were prepared according to the supplier's recommendations. For the CHROMagar Enterobacteria medium, the media were either prepared on the day of analysis and used as-required without adding the supplements, or prepared 24 to 48 hours before analysis and used with the addition of the two supplements.

### III.2. Sample preparation and inoculation

The duplicate samples from the laboratory were thawed before analysis.

For liquid samples, 1 ml of the product was inoculated onto a Petri dish (dilution 0). For solid samples, the test sample (10–25 g depending on the sample) was diluted to 1:2 or 1:10 in tryptone salt (TS). The stock suspension was diluted if necessary to 1:5 or 1:10 in TS.

A volume of 1 ml of each dilution was inoculated in parallel on the two media. Enumerations on both media were therefore made using the same dilution.

### III.3. Incubation and confirmation of colonies

The media were incubated at 37°C for 24 hours. Incubation was then continued to 48 hours.

After incubation, the typical colony types were counted:

- on the VRBG medium: purple colonies
- on the CHROMagar Enterobacteria medium: blue colonies presumed *E. coli* and pink colonies (other enterobacteria), *Proteus* appears in brown.

For 11 samples for which discrepancies were observed between results on the two media, identifications were performed on 1 to 3 isolated colonies from the CHROMagar for 10 samples and from the VRBG for 2 samples.

Identifications was carried out by Gram stain and an Oxidase assay to differentiate *Enterobacteriaceae* from *Pseudomonadaceae*. Identification of species was carried out using the Bruker Biotyper system (MALDITOF mass spectrometer).

## ■ IV. RESULTS

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69 samples were analysed. 50 samples were positive (enumeration  $\geq 10$  CFU/g) after 24 hours of incubation (a 51st sample was positive on VRBG after 48 hours of incubation).

Of the 69 samples analysed, 44 were positive after 24 hours on the VRBG medium and 48 on CHROMagar.

### IV.1. Interpretation of results after 24 hours incubation at 37°C.

For the CHROMagar medium, all red and blue colonies were taken into account (Table 3).

- For 19 samples, the enumerations are  $< 10$  CFU/g for both VRBG and CHROMagar™ Enterobacteria media after 24 hours of incubation (one sample  $> 10$  CFU/g on VRBG after 48 hours).
- 4 samples (cream, bacon morcels, chipolatas, roast chicken sandwich) have very low contamination, at the limit of detection:
  - o for 2 of these samples, the enumeration is below the limit of detection on VRBG whereas 1 to 2 typical colonies were counted on the CHROMagar medium (2 CFU/g for cream and 10 CFU/g for bacon morcels),
  - o for 2 of these samples, the enumeration is lower than the limit of detection on CHROMagar whereas 1 typical colony was counted on VRBG (10 CFU/g for the chipolatas and the roast chicken sandwich).
- For 28 samples, the enumerations were comparable between VRBG and CHROMagar (difference less than 0.5 log).
- For 1 sample (Norwegian smoked salmon), the enumeration was significantly higher (difference  $> 0.5$  log) on VRBG: 50 CFU/g, versus 10 CFU/g on CHROMagar.
- For 17 samples, the enumeration is significantly higher ( $> 0.5$  log difference) on CHROMagar:
  - o for 4 samples, the enumeration is below the threshold of 10 CFU/g on VRBG and varies according to the sample of 30 and 210 CFU/g on CHROMagar,
  - o for 13 samples, the enumerations are greater than the threshold of quantification for the two media with significantly higher enumerations on CHROMagar (differences between the two media ranging from 0.53 to 1.77 log- see Annex 2).

Taking into consideration the 50 positive samples, there is agreement between the two methods for 32 samples (64%). For 1 sample (2%), the enumeration is significantly higher on VRBG. For 17 samples (34%), the enumeration is significantly higher on CHROMagar. There is no link between the method of preparation of the medium (extemporaneously or not) or between the category of foodstuff and the discrepancies observed.

Note: a significant difference between the two media is considered to be a difference greater than 0.5 log.

## IV.2. Confirmation of colonies

A total of 28 isolates were identified (Table 3):

- 3 isolated colonies from the VRBG medium having an enumeration greater than that obtained on the CHROMagar Enterobacteria medium. The 3 strains are confirmed as belonging to the Enterobacteria, with the species *E. coli* (chipolatas) and *Pantoea agglomerans* (Norwegian salmon).
- 1 isolated colony from the CHROMagar medium with a lower enumeration than on VRBG: *Serratia liquefaciens* (Norwegian salmon).
- 24 isolated colonies from the CHROMagar medium with an enumeration higher than that obtained on CHROMagar Enterobacteria medium,
  - o For two samples (cod and batavia lettuce), two out of three colonies were identified as *Pseudomonas* and other related genera.
  - o The other colonies were confirmed as belonging to the Enterobacteria, with the following species present: *Serratia liquefaciens*, *Serratia grimesii*, *Serratia marscescens* (4 samples: cream, 2 stuffed veal parcels, smoked salmon), *Hafnia alvei* (cod and Scottish smoked salmon), *Enterobacter cloacae* (cream cheese), *Rahnella aquatilis* (herring and potatoes in oil), *Pantoea agglomerans* (batavia lettuce), *Moellerella wisconsensis* (bacon morcels) nature), *Erwinia persicina* (cream), *E. coli* (one isolated blue colony from a stuffed veal parcel).

These species are commonly found in the environment and in food.

- *E. coli*, *Hafnia alvei*, *Enterobacter cloacae* and *Moellerella wisconsensis* are intestinal bacteria that can be found in the environment and in foods (dairy products, foods of animal or vegetable origin).
- *Rahnella aquatilis* is isolated from water and soil and is present in certain animals. This species is often isolated from plants.
- *Serratia* are isolated from species, plants, digestive tract, rodents, insects, water and soil.
- *Pantoea agglomerans* is ubiquitous and commonly isolated from plants and animal faeces
- *Erwinia persicina* is a phytopathogenic species commonly isolated from plants (fruits, leguminous vegetables).

Note: all MALDI-TOF identifications provided a very good score (identification sure) except for *Erwinia persicina* strain (identification doubtful)

**Table 3 results in CFU/ml or CFU/g** (the result at 48 hours is shown only when it differs from that at 24 hours, <sup>(1)</sup> double sample)

Products	Description	Enumeration on VRBG in CFU/g or /ml		Enumeration on CHROMagar Enterobacteria in CFU/g or /ml		comments
		24 H	48 H	24 H	48 H	
Samples <quantification threshold for both media after 24 hours of incubation (19)	Meringue and custard dessert	< 10		< 10		
	Egg custard	< 10		< 10		
	Poultry frankfurter	< 10		< 10		
	Sliced chicken fillet	< 10		< 10		
	Sliced braised ham	< 10		< 10		
	Thin bacon morcels	< 10		< 10		
	Cured sausage	< 10		< 10		
	Minced beef	< 10		< 10		
	Smoked salmon morcels	< 10		< 10		
	Smoked trout	< 10		< 10		
	Cooked shrimps	< 10		< 10		
	Chocolate cream pastry dessert	< 10		< 10		
	Raspberry tart	< 10		< 10		
	Pizza 3 cheeses	< 10		< 10		
	Ham and egg salad	< 10		< 10		
	Savoury semolina	< 10		< 10		
	Greek mushroom dish	< 10		< 10		
	Apple, cheese and tripe sausage salad	< 10		< 10		
	Egg and ham sandwich	< 10	10	< 10	< 10	
Samples close to the threshold of quantification for both media (4)	Cream <sup>(1)</sup>	< 1		2		1 colony = <i>Serratia marcescens</i> 1 colony = <i>Erwinia persicina</i> (CHROMagar)
	Bacon morcels	< 10		10		<i>Moellerella wisconsensis</i> (CHROMagar)
	Roast chicken sandwich	10		< 10		
	Chipolatas	10		< 10		<i>E. coli</i> (VRBG)



Products	Description	Enumeration on VRBG in CFU/g or /ml		Enumeration on CHROMagar Enterobacteria in CFU/g or /ml		comments
		24 H	48H	24H	48H	
Samples above the threshold of quantification for both media with comparable results (28)	Crottin de Chavignol cheese	1,080		10 blue 1,480 red		Numerous small colonies on VRBG (not enumerated)
	Soft cheese, brand Livarot	1.6x10 <sup>7</sup>		3.1x10 <sup>7</sup>		
	Pasteurised camembert	1.1x10 <sup>7</sup>		2.2x10 <sup>7</sup>		
	Minced beef, butcher	240		370		
	Kidney (pork)	360		580		
	Chicken leg with skin	50		20 blue 60 red		
	Turkey breast	380		480 blue 120 red		
	Turkey breast	2040		150 red 860 blue		
	Ham	150	300	260	320	
	Sliced ham	120		320		
	Ham with rind	270		390		
	Sausages with herbs	660		460		
	Sausage	1,620		1,600 red 90 blue		
	Chipolatas	950		890		
	Danish smoked salmon	40		30		
	Salmon steak	620		820		
	Pollock	130	220	160	380 red 40 blue	
	Pouting	740	1,220	230 blue 680 red	300 blue 1,370 red	
	Pizza with goat cheese	10		10		
	Chicken caesar wrap	10		20		
	Ham sandwich with salad and mozzarella	330	410	460	540	
	Red cabbage	4,000		4,000		
	Grated carrots	1.1x10 <sup>6</sup>		3x10 <sup>6</sup>		
	Curly Lettuce	1,000		4,000		
Variegated lettuce	7,920		6,000			
Lettuce	1,340		3,000			
Stuffed veal parcels 175	390		620	740		
Heart (beef)	790		10 blue 1,160 red			
Sample with a significantly higher enumeration on VRBG (1)	Norwegian smoked salmon	50	50	10	30	2 colonies = <i>Pantoea agglomerans</i> (VRBG) 1 colony = <i>Serratia liquefaciens</i> (CHROMagar)

Products	Description	Enumeration on VRBG in CFU/g or /ml		Enumeration on CHROMagar Enterobacteria in CFU/g or /ml		comments
		24 H	48H	24H	48H	
Samples with a significantly higher enumeration on CHROMagar (17)	Scottish smoked salmon	< 10		30		3 colonies = <i>Hafnia alvei</i> (CHROMagar)
	Toulouse sausage	< 10		50		
	Stuffed veal parcel <sup>(1)</sup>	< 10		90		3 colonies = <i>Serratia liquefaciens</i> (CHROMagar)
	Herring potato in oil	< 10	110	210	1,440	3 colonies = <i>Rahnella aquatilis</i> (CHROMagar)
	cod	10	50	30	150	2 colonies = <i>Pseudomonas</i> 1 colony = <i>Hafnia alvei</i> (CHROMagar)
	Curdled milk <sup>(1)</sup>	6	14	32	+ very small colonies	
	Cream cheese	10		110		3 colonies = <i>Enterobacter cloacae</i> (CHROMagar)
	Goat cheese wrap	10	10	590	630	
	Batavia lettuce	10		110		2 colonies = <i>Pseudomonas</i> 1 colony = <i>Pantoea agglomerans</i> (CHROMagar)
	Almond and cream pastry dessert	10	10	50	70	
	Strawberry tart	10	10	110	150	
	Whiting fillet	30		130	150	
	Organic stuffed veal parcel 310	90		300 dt 50 blue	360 dt 50 blue	2 red colonies = <i>Serratia liquefaciens</i> , <i>Serratia grimesii</i> 1 blue = <i>E. coli</i> (CHROMagar)
	Minced beef	2x10 <sup>3</sup>		1.2x10 <sup>4</sup>	1.4x10 <sup>4</sup>	
	Tunisian spiced sausage	2x10 <sup>3</sup>		1.5x10 <sup>4</sup> red 60 blue	1.5x10 <sup>4</sup> red 70 blue	
	Liver (beef)	3x10 <sup>3</sup>		60 blue 1.5x10 <sup>4</sup> red	60 blue 3.7x10 <sup>4</sup> red	
	Soft cheese, brand Pont l'Eveque	2.5x10 <sup>6</sup>		9.6x10 <sup>6</sup>		

## ■ V. CONCLUSION

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69 samples of food products of various origins were analysed **according to norm NF V 08-054** on CHROMagar medium and seeded in a **double layer using pouring method** in VRBG medium and **incubated at 37°C**.

Of the 69 samples analysed, 44 were positive after 24 hours on the VRBG medium and 48 on CHROMagar. A total of 50 samples were positive (enumeration  $\geq 10$  CFU/g) after 24 hours of incubation.

Taking into consideration the 50 positive samples, there is agreement between the two methods for 32 samples (64%). For 1 sample (2%), the enumeration is significantly higher on VRBG. For 17 samples (34%), the enumeration is significantly higher on CHROMagar. Identifications confirmed that these were Enterobacteriaceae with the exception of two samples, for which the enumeration is overestimated due to the quantification of *Pseudomonas*.

The CHROMagar Enterobacteria medium has the advantage of distinguishing isolates from *E. coli* species.

## APPENDIX 1 data sheet of the CHROMagar Enterobacteria medium

### CHROMagar™ Enterobacteria

Chromogenic medium for detection and enumeration of Enterobacteria

Composed by :

CHROMagar™ Enterobacteria base: ref. X214B-5

#### ONLY FOR THE BOTTLE TECHNIQUE :

CHROMagar™ Enterobacteria sup S1 : ref. X214S1

CHROMagar™ Enterobacteria sup S2 : ref. X214S2 (box of 10 vials ref. X214S2-500)

#### STORAGE :

Store the powder base until the shelflife date indicated on the labels as following :

ref. X214B → 15/30°C

ref. X214S1 → 2/8°C

ref. X214S2 → 2/8°C

#### COMPOSITION in g/L :

Base → Agar 11.0; Peptones and yeast extract 22.0; Mineral salts 4.9; Chromogenic and selective mix 0.7; Growth factors 3.7. Total 42.3 g/L  
Supplement S1 → chromogenic mix 1ml/L  
Supplement S2 → selective mix 0,01

pH : 7.4 ± 0.2 (Classical formula adjusted and/or supplemented as required to meet performance criteria).

#### PREPARATION

(Calculation for 1L of final media) :

→ BASE ref X214B

• Suspend the powder base in the proportion of 42.3g into 1L of purified water.

• Disperse powder slowly in water by rotating for swelling of the agar. Swirl for mixing.

• Heat and bring to boiling (100°C) while swirling or stirring regularly. If using an autoclave, do so without pressure. DO NOT HEAT TO MORE THAN 100°C. The 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 keeping stirring.

#### INOCULATION :

• If using surface technique procedure:

Pour into sterile Petri dishes and allow to gel and dry.

If the agar plate has been refrigerated, allow to warm to room temperature before inoculation. Streak sample onto plate and incubate at 37°C for 24 hours.

• Note : Prepared media plates can be kept for one day at ambient temperature or for up to one month under refrigeration (2/8°C) if properly prepared and protected from light and dehydration.

• If using pouring technique procedure:

Prepare 90mm Ø sterile Petri dishes and add 1ml of inoculum in each. Then pour 10ml of melted medium. Mix and let solidify. Add an overlay of the same medium and let solidify. Invert and incubate at 37°C for 24 hours.

• If using pouring technique procedure with utilisation of 250ml prepared bottle:

• Pour into bottles and let solidify.

• Note : Prepared media bottles can be kept for one day at ambient temperature or for up to one week under refrigeration (2/8°C) if properly prepared and protected from light and dehydration.

• Heat the bottle containing the solidified CHROMagar Enterobacteria base at 100°C until complete melting of the media (30 minutes to 2 hours). If using an autoclave, do so without pressure. DO NOT HEAT TO MORE THAN 100°C.

• Cool in a water bath to 45/50°C.

• Add before pouring 250µl of CHROMagar Enterobacteria supplement S1.

• Rehydrate one freeze dried vial of CHROMagar Enterobacteria supplement S2 with 6ml of sterile water and add 3ml of this rehydrated freeze dried vial into the bottle.

• Stir well the bottle

• Prepare 90mm Ø sterile Petri dishes and add 1ml of inoculum in each. Then pour 10ml of melted medium. Mix and let solidify. Add an overlay of the same medium and let solidify. Invert and incubate at 37°C for 24 hours.

Note : Reconstituted CHROMagar Enterobacteria supplement S2 solution can be stored for up to two weeks under refrigeration (2/8°C) and at -20°C.

#### INTERPRETATION :

This selective medium is inhibitory for many microorganisms, mostly gram positive.

#### Microorganism → Typical colony appearance

*E.coli* → Blue with or without blue halo

Other enterobacteria → Pink

*Proteus* → Red with swarming

*Pseudomonas* → Mostly inhibited

Gram positive → Inhibited

#### PERFORMANCE and LIMITATIONS :

Definite identification may require additional testing.

Indole Test can be done to confirm *E.coli*.

Oxydase test can be done to eliminate possible *Pseudomonas*.

#### DISPOSAL OF WASTE :

After interpretation all plates should be destroyed by autoclaving at 121°C for at least 20 minutes.

For Research Use Only. Laboratory product to be used only by trained personnel.

Comments to scientists testing this trial formula : we need feedback about this trial formula. We would suggest several tests:

- 1) testing pure strains from your collections of Enterobacteria in order to assay the sensitivity (how many false negative ?) and testing pure strains from your collection of non- Enterobacteria in order to assay the specificity (how many false positive ?) *It would be very kind of you if you could send us some false negative strains and some false positive strains from collections.*
- 2) testing real samples in order to assay the positive predictive value (related to false positive samples) in order to assay the negative predictive value (related to false negative samples). *It would be very kind of you if you could send us some false negative strains and some false positive strains from samples.*

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(7/2014)

version 1

CHROMagar™ is a trademark created by Dr. A. Rambach

**APPENDIX 2 results expressed in log CFU/g for samples with discrepancies**

	VRBG medium		CHROMagar medium		enumeration difference in log
	CFU/g	CFU log/g	CFU/g	CFU log/g	
Norwegian smoked salmon	50	1.7	10	1	0.70
Curdled milk	6	0.78	32	1.51	0.73
Cream cheese	10	1	110	2.04	1.04
Batavia lettuce	10	1	110	2.04	1.04
Tomato wrap	10	1	590	2.77	1.77
Almond and cream pastry dessert	10	1	50	1.70	0.70
Strawberry tart	10	1	110	2.04	1.04
Whiting fillet	30	1.48	130	2.11	0.64
Organic stuffed veal parcel	90	1.95	300	2.48	0.53
Minced beef	2,000	3.3	1.2x10 <sup>4</sup>	4.08	0.78
Tunisian spiced sausage	2,000	3.3	1.5x10 <sup>4</sup>	4.18	0.88
Liver (beef)	3,000	3.3	1.5x10 <sup>4</sup>	4.18	0.88
Soft cheese, brand Pont d'Eveque	2.5x10 <sup>6</sup>	6.4	9.6x10 <sup>6</sup>	6.98	0.58