Summary report 2010LR35

PRINCIPLE OF THE METHOD

The ChromID™ L. mono Agar consists of a nutritive base combining different peptones and a chromogenic substrate. The differentiation of *Listeria monocytogenes* from other species of *Listeria* is based on the appearance of typical blue-turquoise colonies. The selective mixture inhibits the growth of most other bacteria and yeasts.

Samples diluted in buffered peptone water or in peptone salt are inoculated onto ChromID Lmono plates. Both the pour plate method and the surface inoculation method were tested and for each of them the following parameters were tested:

- Surface plating (Ref 43661):
    - for the primary dilution, plating of 1 ml on 3 plates and of 1 ml on 2 plates,
    - counting and confirmation of the plates after an incubation period of 24 hours and 48 hours (minimum and maximum of the proposed incubation interval),
    - counting and confirmation after a storage post incubation of the plates, for 72 hours at 2-8°C.
- Pour plating (Ref 42661):
    - counting and confirmation of the plates after an incubation period of 26 hours and 48 hours (minimum and maximum of the proposed incubation interval),
    - counting and confirmation after a storage post incubation of the plates, for 72 hours at 2-8°C.

For both plating protocols, characteristic colonies were confirmed using all the following proposed confirmatory tests:

- Confirmatory tests described in the ISO 11290-2 (1998) on one purified colony (the characteristic colony observed on ChromID Lmono medium was purified on a nutritive agar plate before performing the confirmatory tests);
- RAPIDEC gallery on an isolated colony observed on ChromID Lmono medium, without purification step;
- API gallery on an isolated colony observed on ChromID Lmono medium, without purification step;
- VIDAS LMO2 on a colony observed on ChromID Lmono medium (isolated or not), without purification step;
- Accuprobe LMO on a colony observed on ChromID Lmono medium, (isolated or not, without purification step.

SCOPE

All food categories & environmental samples

RESTRICTION OF USE

None

REFERENCE METHOD

ISO 11290-2 (1998) & ISO 11290-2/A1 (2004): Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 2: enumeration of *Listeria monocytogenes* in foods

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LINEARITY and RELATIVE ACCURACY

Comparison of the performance of the alternative method and the reference method was conducted.
Note : All the confirmatory tests and the sample suspension broths were tested.

Linearity study

The study performed in 2010 included 33 artificially contaminated samples. The five categories were represented with the following matrices: raw pork minced meat, blue cheese, frozen vegetables, smoked salmon and process water.

Five levels of contamination within the ranges: 10^2 CFU/g, 5.10^2 CFU/g, 10^3 CFU/g, 5.10^3 CFU/g, 10^4 CFU/g and two samples per level were analyzed by both methods. The results are presented according to all the tested protocols, i.e. pour and surface plating, incubation time, confirmatory tests.

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Table of results : Comparison of the ChromID Lmono Agar (LMO-F) method with the ISO 11290-2 (1998) & ISO 11290-2/A1 (2004) method:

Matrix	Inoculation method	Interpretation	R	Selected regression	Rob.F	Critical value	P%	Correlation coefficient	Regression equation
Raw minced meat	Plating	24H-2plates	1,25	GMFR	4,1	3,58	4	0,9974	log Alt=1,0298 log Ref-0,1249
		24H-3 plates	2,13	OLS1	0,57	3,58	75	0,9936	log Alt=1,0118 log Ref-0,0759
		48H-2 plates	1,25	GMFR	4,092	3,58	4	0,9974	log Alt=1,0273 log Ref-0,1147
		48H-3 plates	2,13	OLS1	0,434	3,58	84	0,994	log Alt=1,0102 log Ref-0,0688
		72H-2 plates	1,25	GMFR	4,092	3,58	4	0,9974	log Alt=1,0273 log Ref-0,1147
		72H-3 plates	2,13	OLS1	0,434	3,58	84	0,994	log Alt=1,0102 log Ref-0,0688
	Inclusion	26H	1,62	GMFR	14,871	3,58	0	0,9842	log Alt=0,944 log Ref+0,0815
		48H	2,50	OLS1	2,685	3,58	10	0,9785	log Alt=0,933 log Ref+0,1195
		72H	2,50	OLS1	2,685	3,58	10	0,9785	log Alt=0,933 log Ref+0,1195
Blue cheese	Plating	24H-2plates	0,55	GMFR	19,783	5,41	0	0,9898	log Alt=1,0721 log Ref-0,1881
		24H-3 plates	0,27	OLS2	0,704	5,41	59	0,9885	log Ref=0,9279 log Alt+0,1866
		48H-2 plates	0,55	GMFR	19,783	5,41	0	0,9898	log Alt=1,0721 log Ref-0,1881
		48H-3 plates	0,27	OLS2	0,818	5,41	54	0,988	log Ref=0,9264 log Alt+0,1879
		72H-2 plates	0,55	GMFR	19,783	5,41	0	0,9898	log Alt=1,0721 log Ref-0,1881
		72H-3 plates	0,27	OLS2	0,818	5,41	54	0,988	log Ref=0,9264 log Alt+0,1879
	Inclusion	26H	0,73	GMFR	5,367	5,41	5	0,994	log Alt=1,0697 log Ref-0,2668
		48H	0,55	GMFR	11,171	5,41	1	0,994	log Alt=1,0788 log Ref-0,2839
		72H	0,55	GMFR	11,171	5,41	1	0,994	log Alt=1,0788 log Ref-0,2839
Frozen vegetables	Plating	24H-2plates	1,00	GMFR	6,453	3,97	1	0,9961	log Alt=0,9586 log Ref+0,0555
		24H-3 plates	2,50	OLS1	0,176	3,97	98	0,9871	log Alt=0,9072 log Ref+0,2409
		48H-2 plates	2,00	GMFR	0,237	3,97	93	0,9966	log Alt=0,9408 log Ref+0,1300
		48H-3 plates	2,25	OLS1	0,032	3,97	100	0,99	log Alt=0,8842 log Ref+0,3322
		72H-2 plates	2,00	GMFR	0,188	3,97	96	0,9967	log Alt=0,9408 log Ref+0,1314
		72H-3 plates	2,25	OLS1	0,000	3,97	100	0,9904	log Alt=0,8812 log Ref+0,3432
	Inclusion	26H	1,50	GMFR	3,548	3,97	6	0,9945	log Alt=0,9579 log Ref-0,0233
		48H	1,00	GMFR	12,459	3,97	0	0,9933	log Alt=0,9744 log Ref-0,0574
		72H	1,00	GMFR	12,459	3,97	0	0,9933	log Alt=0,9744 log Ref-0,0574
Smoked salmon	Plating	24H-2plates	0,88	GMFR	5,1371	3,97	3	0,9974	log Alt=1,0056 log Ref-0,0453
		24H-3 plates	0,63	GMFR	10,408	3,97	0	0,9975	log Alt=0,9773 log Ref+0,0795
		48H-2 plates	1,50	GMFR	0,388	3,97	84	0,9979	log Alt=0,9992 log Ref-0,0173
		48H-3 plates	0,63	GMFR	11,267	3,97	0	0,9973	log Alt=0,9735 log Ref+0,0953
		72H-2 plates	1,50	GMFR	0,388	3,97	84	0,9979	log Alt=0,9992 log Ref-0,0173
		72H-3 plates	0,63	GMFR	11,267	3,97	0	0,9973	log Alt=0,9735 log Ref+0,0953
	Inclusion	26H	1,00	GMFR	3,902	3,97	5	0,997	log Alt=0,9547 log Ref+0,0952
		48H	1,00	GMFR	4,957	3,97	3	0,9963	log Alt=0,94652 log Ref+0,1302
		72H	1,00	GMFR	4,957	3,97	3	0,9963	log Alt=0,94652 log Ref+0,1302
Process water	Plating	24H-2plates	3,60	OLS1	0,000	4,53	100	0,9974	log Alt=1,0173 log Ref-0,1526
		24H-3 plates	4,60	OLS1	0,000	4,53	100	0,9963	log Alt=1,0310 log Ref-0,2077
		48H-2 plates	2,60	OLS1	2,476	4,53	25	0,9957	log Alt=0,9808 log Ref+0,0759
		48H-3 plates	2,20	OLS1	2,949	4,53	11	0,9967	log Alt=1,0002 log Ref+0,,0026
		72H-2 plates	3,00	OLS1	1,833	4,53	24	0,9952	log Alt=0,9840 log Ref+0,0723
		72H-3 plates	2,20	OLS1	3,554	4,53	8	0,9962	log Alt=1,0032 log Ref-0,0012
	Inclusion	26H	2,60	OLS1	0,700	4,53	62	0,9979	log Alt=1,0405 log Ref-0,3900
		48H	3,00	OLS1	0,000	4,53	100	0,9981	log Alt=0,9973 log Ref-0,1149
		72H	3,00	OLS1	0,067	4,53	99	0,9978	log Alt=0,9950 log Ref-0,0960

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The linearity of the ChromID Lmono Agar (LMO-F) was assessed in all the tested cases for *Listeria monocytogenes* enumeration in all human food products and environmental samples.

Relative accuracy study

In addition to the data obtained in the linearity study, fifty samples covering the five categories i.e. raw pork minced meat, blue cheese, frozen vegetables, smoked salmon and process water were analysed in duplicate by both methods. Of these samples, 54 including 34artificially contaminated samples gave interpretable results.

The contaminated levels were comprised between:

Category	Contamination range (in log CFU/g)
Meat and meat products	1,40 to 3,93
Dairy products	1,60 to 3,75
Seafood	1,78 to 4,82
Fruits and vegetables	1,90 to 5,88
Environmental samples	1,78 to 4,79

Overall, the equations of the regression lines between the alternative method and the reference method were the following according to the tested protocols:

Matrix	Inoculation method	Interpretation	n	R	Selected regression	a	t(a)	b	t(b)	T critical value	P%		Bias
											Intercept (0)	Slope(1)	
Meat products	Plating	24H-2plates	14	1,17	GMFR	0,007	0,088	0,991	0,320	2,179	93	75	-0,018
		24H-3 plates	14	1,29	GMFR	0,130	1,624	0,952	1,604	2,179	13	13	-0,010
		48H-2 plates	14	0,92	GMFR	0,129	1,283	0,955	1,181	2,179	22	26	-0,008
		48H-3 plates	15	1,29	GMFR	0,143	1,831	0,949	1,698	2,160	9	11	-0,005
		72H-2 plates	14	0,92	GMFR	0,129	1,283	0,955	1,181	2,179	22	26	-0,008
		72H-3 plates	15	1,29	GMFR	0,143	1,831	0,949	1,698	2,160	9	11	-0,005
	Inclusion	26H	13	1,29	GMFR	0,170	1,453	0,919	1,869	2,201	17	9	-0,030
		48H	13	1,29	GMFR	0,181	1,585	0,919	1,917	2,201	14	8	-0,030
		72H	13	1,57	GMFR	0,207	1,783	0,911	2,076	2,201	10	6	-0,030
Dairy products	Plating	24H-2plates	10	0,94	GMFR	0,299	0,769	0,889	0,821	2,306	46	44	0,010
		24H-3 plates	10	0,82	GMFR	0,323	0,823	0,875	0,917	2,306	43	39	-0,007
		48H-2 plates	10	0,71	GMFR	0,296	0,887	0,898	0,879	2,306	40	40	0,010
		48H-3 plates	10	0,94	GMFR	0,281	0,892	0,907	0,855	2,306	40	42	0,025
		72H-2 plates	10	0,71	GMFR	0,296	0,887	0,898	0,879	2,306	40	40	0,010
		72H-3 plates	10	0,94	GMFR	0,281	0,892	0,907	0,855	2,306	40	42	0,025
	Inclusion	26H	10	0,94	GMFR	0,144	0,402	0,926	0,593	2,306	70	57	-0,055
		48H	10	1,18	GMFR	0,130	0,410	0,938	0,561	2,306	69	59	-0,055
		72H	10	1,18	GMFR	0,130	0,410	0,938	0,561	2,306	69	59	-0,055

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Matrix	Inoculation method	Interpretation	n	R	Selected regression	a	t(a)	b	t(b)	T critical value	P%		Bias
Seafood	Plating	24H-2plates	16	2,33	OLS1	-0,208	0,786	1,023	0,294	2,145	45	77	-0,103
		24H-3 plates	16	1,83	GMFR	-0,218	0,876	1,034	0,447	2,145	40	66	-0,065
		48H-2 plates	16	2,17	GMFR	-0,086	0,785	1,014	0,427	2,145	68	45	-0,015
		48H-3 plates	16	1,67	GMFR	0,002	0,019	0,992	0,217	2,145	98	83	-0,013
		72H-2 plates	16	2,00	GMFR	-0,076	0,708	1,012	0,363	2,145	49	72	-0,015
		72H-3 plates	16	1,83	GMFR	0,007	0,060	0,991	0,244	2,145	95	81	-0,013
	Inclusion	26H	16	2,50	OLS1	-0,022	0,112	0,967	0,565	2,145	91	58	-0,093
		48H	16	2,50	OLS1	0,089	0,571	0,954	0,974	2,145	58	35	-0,080
		72H	16	2,50	OLS1	0,089	0,571	0,954	0,974	2,145	58	35	-0,080
Environmental samples	Plating	24H-2plates	12	1,46	GMFR	-0,169	2,638	1,024	1,250	2,228	2	24	-0,098
		24H-3 plates	12	1,23	GMFR	-0,142	1,247	1,018	0,524	2,228	24	61	-0,103
		48H-2 plates	12	1,08	GMFR	-0,048	0,448	1,005	0,171	2,228	66	87	-0,018
		48H-3 plates	12	1,46	GMFR	-0,040	0,351	0,997	0,105	2,228	73	92	-0,075
		72H-2 plates	12	1,08	GMFR	-0,032	0,280	1,002	0,067	2,228	79	95	-0,018
		72H-3 plates	12	0,85	GMFR	0,041	0,434	0,989	0,413	2,228	67	69	-0,010
	Inclusion	26H	12	1,00	GMFR	-0,351	2,217	1,040	0,846	2,228	5	42	-0,215
		48H	12	1,23	GMFR	-0,147	0,926	1,006	0,125	2,228	38	90	-0,130
		72H	12	1,23	GMFR	-0,136	0,838	1,005	0,096	2,228	42	93	-0,125
All products	Plating	24H-2plates	70	1,36	GMFR	-0,076	0,967	0,998	0,074	1,995	34	94	-0,067
		24H-3 plates	70	1,42	GMFR	-0,007	0,092	0,981	0,785	1,995	93	44	-0,048
		48H-2 plates	70	1,27	GMFR	0,025	0,450	0,984	0,933	1,995	65	35	-0,015
		48H-3 plates	71	1,33	GMFR	0,059	1,154	0,976	1,509	1,995	25	14	-0,020
		72H-2 plates	70	1,27	GMFR	0,025	0,458	0,985	0,897	1,995	65	37	-0,015
		72H-3 plates	71	1,17	GMFR	0,064	1,283	0,977	1,450	1,995	20	15	-0,015
	Inclusion	26H	69	1,33	GMFR	-0,047	0,620	0,976	1,049	1,995	54	30	-0,100
		48H	69	1,33	GMFR	0,010	0,159	0,974	1,295	1,995	87	20	-0,080
		72H	69	1,33	GMFR	0,018	0,271	0,972	1,373	1,995	79	17	-0,080

Conclusion: ChromID Lmono agar method showed a good correlation to the reference method, indicating equivalence to ISO 11290-2 (1998) & ISO 11290-2/A1 (2004) for *Listeria monocytogenes* enumeration in all human food products and environmental samples.

SELECTIVITY (INCLUSIVITY/EXCLUSIVITY)

30 strains were tested. Whatever the tested plating protocol (surface or pour) and incubation time, all the strains gave typical colonies with the ChromID Lmono agar: green to blue colonies.
20 non target strains were tested, including 12 strains belonging to the Listeria genus and different from the L. monocytogenes species; some of *L. ivanovii* strains gave characteristic blue colonies on the ChromID Lmono agar, but the same result was observed with the ISO11290-2 method.

The ChromID Lmono agar method shows satisfactory inclusivity and exclusivity performances.

PRACTICABILITY (Alternative Method only)

The ChromID Lmono method is a new *Listeria monocytogenes* enumeration method based on a chromogenic selective agar. The users can choose the plating protocol (pour or surface), as well as the confirmatory tests they want to realize in their routine analyses. Final results are obtained the next day by using a Rapidec gallery, the VIDAS LMO2 assay or the AccuProbe Lmono assay as confirmatory tests.

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INTERLABORATORY STUDY

The inter-laboratory study was conducted in December 2010 with 14 laboratories in 4 European countries. Samples of cream cheese with walnuts were artificially contaminated with a single strain (*Listeria monocytogenes* 153) to provide samples with the following contamination levels; low (40 to 100 CFU/ml), medium (100 to 1 000 CFU/ml) and high (1 000 to 10 000 CFU/ml). Uninoculated samples were used to provide the forth contamination level (0 CFU/ml). Each laboratory received duplicate blind-coded samples for each contamination level, which were tested by both methods.

Results obtained:

Contamination level	Number of samples	Reference method		Alternative method		
		Repeatability sd	Reproducibility sd	Repeatability sd	Reproducibility sd	Bias
Low	96	0,1023	0,1067	0,1602	0,1602	- 0,0636
Medium	96	0,0662	0,0934	0,1032	0,1032	- 0,0026
High	96	0,0870	0,1106	0,0866	0,1097	0,0060

On the criteria of bias, repeatability and reproducibility, equivalent results were obtained by both tested methods, i.e. chromID Lmono and the ISO 11290-2 methods.

CONCLUSION

The method comparison study and the interlaboratory study showed comparable results between the ChromoID Lmono agar method and the ISO 112902-2 and 11290-2/A1 method for enumeration of *Listeria monocytogenes* in all foods.

Please send any queries concerning the performance of the validated method to Lloyd's Register Quality Assurance.

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