Summary report 2010LR38

PRINCIPLE OF THE METHOD

CASA™ method is a chromogenic culture medium for the enumeration of *Campylobacter* spp in meat, poultry products and environmental samples. It consists of a nutritive base combining different peptones and a chromogenic substrate. The differentiation of *Campylobacter* spp from other species is based on the appearance of typical brick red colonies. The selective mixture inhibits the growth of most of the other micro-organisms.

Samples diluted in buffered peptone water or in peptone salt are inoculated onto CASA™ plates by surface plating. Characteristic colonies are confirmed using all the following proposed confirmation tests:

- Tests described in the ISO/TS 10272-2 (2006);
- Microgen Campylobacter latex test on one characteristic colony

In order to improve the practicability of the method, the reading of the plates and the latex test can be realized after 72H storage at 2-8°C.

SCOPE

Meat products, poultry products & environmental samples

RESTRICTION OF USE

None

REFERENCE METHOD

ISO/TS 10272-2 : 2006 Microbiology of food and animal feeding stuffs. Horizontal method for detection and enumeration of Campylobacter spp. Part 2: Colony count technique

LINEARITY and RELATIVE ACCURACY

Comparison of the performance of the alternative method and the reference method was conducted.

Note : All the confirmatory tests were tested.

Linearity study

The study performed 2010-2011 included 16 artificially contaminated samples. The three studied categories were represented with the following matrices: sausage meat, poultry neck meat and process water.

Five to six levels of contamination were tested, between 50 and 10 0000 CFU/g. Two samples were analyzed per level of contamination by both methods.

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Table of results: Comparison of the CASA™ method with the ISO/TS 10272-2 method:

Matrix	Correlation coefficient	Regression equation
Sausage meat	0,998	Log Alt = 1,114 log Ref. - 0,484
Chicken neck meat	0,994	Log Alt = 0,964 log Ref. + 0,061
Process water	0,945	Log Ref. = 0,910 log Alt. + 0,466

The linearity of the CASA™ method was assessed cases for *Campylobacter* spp enumeration in meat products, poultry products and environmental samples.

Relative accuracy study

In addition to the data obtained in the linearity study, 71 samples covering the three studied categories were analysed in duplicate by both methods. Of these samples, 39 including 25 artificially contaminated samples gave interpretable results.

The contaminated levels were comprised between:

Category	Contamination range (in log CFU/g)
Meat products	1,70 to 4,48
Poultry products	1,48 to 3,64
Environmental samples	1,48 to 3,34

Overall, the equations of the regression lines between the alternative method and the reference method were the following:

Category	Correlation coefficient	Regression equation
Meat and meat products	0,98	Log Alt = 1,023 log Ref. - 0,291
Poultry and poultry products	0,99	Log Alt = 0,962 log Ref. + 0,041
Environmental samples	0,91	Log Alt = 0,950 log Ref. + 0,027
All products	0,96	Log Alt = 0,971 log Ref. - 0,048

The CASA™ method showed a satisfying correlation to the reference method, indicating equivalence to ISO/TS 10272-2 for *Campylobacter* spp enumeration in all meat products, poultry products and environmental samples.

Storage of incubated plates during 72 h at 2-8°C

Results obtained after 44 h± 4h of incubation at 41,5°C were compared to those obtained on same agar plates stored during 72h at 2-8°C in micro-aerobic conditions. The cold storage of agar plates does not modify the results observed just after incubation.

SELECTIVITY (INCLUSIVITY/EXCLUSIVITY)

30 *Campylobacter* strains were tested in the inclusivity study. Characteristic colonies were enumerated for all the tested strains, except for four *Campylobacter upsalienisis* which were not able to grow on CASA™ plates. Note that microscopic colonies or no growth on the tested mCCDA plates were also observed. Three *Campylobacter lari* strains gave negative latex tests.

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26 non target strains were tested in exclusivity study, and were not able to grow or gave non characteristic colonies.
The CASA™ method shows satisfactory inclusivity and exclusivity performances.

PRACTICABILITY (Alternative Method only)

The CASA™ method is a new *Campylobacter* spp enumeration method based on a chromogenic selective agar. The users can choose the confirmatory tests they want to realize in their routine analyses, the ISO/TS 10272-2 confirmation tests or the MicroGen® *Campylobacter* latex test. Final results are obtained in 2 days by using the MicroGen® *Campylobacter* latex test.

INTERLABORATORY STUDY

The inter-laboratory study was conducted in March 2011 with 14 laboratories in 4 European countries. Samples of raw poultry minced meat were artificially contaminated with a single strain (*Campylobacter jejuni* Ad1000) to provide samples with the following contamination levels; low (500 CFU/g), medium (5000 CFU/g) and high (50 000 CFU/g). Non inoculated samples were used to provide the fourth contamination level (0 CFUg). Each laboratory received duplicate blind-coded samples for each contamination level, which were tested by both methods. The results of 11 laboratories were retained for the interpretation, due to some problems encountered during the sample delivery or the carrying out of the analysis. The results are the following:

Level (CFU/g)	Reference method		Alternative method		D (bias)
	Repeatability standard deviation	Reproducibility standard deviation	Repeatability standard deviation	Reproducibility standard deviation	
500	0,1013	0,1332	0,1026	0,1706	-0,0367
5 000	0,1345	0,2069	0,2976	0,3447	-0,0251
50 000	0,0775	0,1906	0,1785	0,2931	-0,1633

On the criteria of bias, repeatability and reproducibility, equivalent results were obtained by both tested methods, i.e. CASA™ and the ISO/TS 10272-2 methods.

CONCLUSION

The method comparison study and the interlaboratory study showed comparable results between the CASA™ method and the ISO/TS 10272-2 method for enumeration of *Campylobacter* spp in meat products, poultry products and environmental samples.

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

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