Assessment of Captivate™ for the detection of *Escherichia coli* O157 in meat, milk, apple juice and cheese

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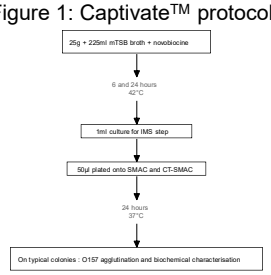
Introduction

Enterohemorrhagic *Escherichia coli* is a serious health problem in various countries. In Belgium, all cases are sporadic and no outbreak has been detected. In the USA and UK, the consumption of poorly cooked minced beef is a major risk factor.
The immunomagnetic Captivate™ method (IDG, England) has been compared with the validated method Dynabeads™ anti-*E. coli* O157 (Dynal, Norway) and the new Probelia™ O157:H7 method (Sanofi Diagnostic Pasteur, France).

Material, Methods and Results

The protocol recommanded by the producer was been followed. Briefly, 25g of tested product is homogenised in 225ml of mTSB broth with novobiocine supplement. After 6 and 24 hours of incubation at 42°C, the immunomagnetic separation step (IMS), which consist essentially, after addition of 20µl of beads to 1ml of culture, in 3 wash steps, is carried out. Fifty µl of the concentrated broth is plated onto sorbitol-Mac Conkey with and without cefixime and tellurite. Both medium are incubated 24 hours at 37°C. After incubation, O157 agglutination and biochemical characterisation are realised on typical colonies (Figure 1).

Figure 1: Captivate™ protocol



Specificity

The specificity was evaluated on pure culture. All strains were cultivated in BHI broth during 24 hours at 37°C. The IMS was directly carried out on the 10^{-8} dilution of these cultures.

Table 1: E. coli strains tested with Captivate™

E. coli tested	Number of strains	Growth on SMAC	Growth on CT-SMAC
O157:H7	21	21	21
non-O157:H7	9	0	0

The Captivate™ method concentrates only strains of *E. coli* O157:H7 and no cross-reactivity occurs with other strains of *E. coli* non O157.

Sensitivity

• Detection limit of the IMS

The detection limit of the IMS was estimated for 2 different strains. Like for specificity, the strains were cultivated 24 hours at 37°C in BHI broth and the IMS realised on 10^{-7} to 10^{-10} dilution. On the other hand,a 10^{-6} dilution was plated onto TSA and incubated 48 hours at 37°C. Both strains were analyzed two times (Table 2).

Table 2: Detection limit of the IMS

Strain	Enumeration	IMS on dilution 10^{-7}	IMS on dilution 10^{-8}	IMS on dilution 10^{-9}
1	$7,0.10^{8}$	+	+	-
1	$4,0.10^{8}$	+	+	-
2	$1,0.10^{8}$	+	+	-
2	$6,0.10^{8}$	+	+	-

• Detection limit of the protocol

Four different matrixes (pasteurized apple juice, pasteurized milk, pasteurized cheese and irradiated meat) were spiked with 0, 1-5, 5-10, 10-20 and 20-50 cfu/25g of product. All spiked samples were analyzed two times (Table 3).

Table 3: Detection limit of the protocol

	Contamination rate (cfu/25g)				
Matrix	0	1-5	5-10	10-20	20-50
Milk	-	not done	not done	+	+
Apple juice	-	not done	not done	+	+
Cheese	-	+	+	+	+
Meat	-	+	+	+	+

The IMS step detects level as low as 1 to 10 cfu *E. coli* O157/ml.
The CaptivateTM method is able to detect *E. coli* O157 in artificially contaminated samples at a rate of 1-5 cfu/25g (irradiated meat and pasteurized cheese) and at a rate lower than 10-25cfu/25g (pasteurized apple juice and pasteurized milk)

Comparison of performance

Meat and meat products were artificially contaminated with *E. coli* O157 at three levels comprise between 5 and 25cfu/25g. All samples were analyzed two times with the three methods (Captivate™ O157, Dynabeads™ anti-*E .coli* O157 and Probelia™O157:H7) and results are shown in table 4.

Table 4 : Comparison of performance in raw meat

	n=	Captivate O157 number of +	Dynal O157 number of +	Probelia O157:H7 number of +
0cfu/25g	1	0	0	0
1-5 cfu/25g	4	4	4	4
5-10 cfu/25g	3	3	3	3
10-25 cfu/g	2	2	2	2

The Captivate™ method is as good as Dynabeads™ and Probelia™ O157:H7 in detecting artificially contaminated raw meat.

Conclusions

•Captivate™ O157 is an efficient method with high specificity and sensitivity. This immunomagnetic separation method should be a very good alternative in order to concentrate *E. coli* O157 directly after preenrichment or to detect the O157 strains in a broth culture after other rapid tests (ELISA, ELFA, PCR, …).
•Further assays will be done on milk and apple juice to assess the real detection limit of the protocol for these matrixes.
•Comparison of performance will be evaluated for milk, apple juice and cheese.
•To determine the practicability of the method, a collaborative trial will be organized.