Comparison of Real Time PCR tests with culture to diagnostic enteropathogens in stool samples

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Background

Infectious gastroenteritis is the most common childhood illnesses worldwide and it is caused by different species of bacteria, viruses and parasites, being *Campylobacter*, *Salmonella* and *Yersinia* three of the main enteropathogens.

The aim of this study is to compare prospectively two different commercial Real-Time PCR assays and establish a simultaneously comparison with the culture method, which is the routine diagnosis technique.

Material/methods

A total of 200 stool samples from patients with gastrointestinal symptoms were cultivated in six different culture medium reflected in ***figure 1***. At the same time, all samples were tested by molecular methods according to ***figure 2*** process. Additionally, discrepant samples for *Campylobacter* were tested by a third Real-Time PCR assay "mericon Campylobacter spp Kit" (Qiagen ®).

Direct detection of *Salmonella* after 24 h of incubation

- MacConkey agar (MCK agar)
- Hektoen enteric agar (HEA)

Direct detection of *Yersinia enterocolitica* after 24 h of incubation

- MacConkey agar (MCK agar)
- Cefsulodina-Irgasan-Novobiocina agar (CIN agar)

Direct detection of *Salmonella* after 48 h of incubation

- Xylose lysine deoxycholate agar (XLD agar)

From selenite broth

Direct detection of *Campylobacter* after 48 h of incubation

- Campylobacter charcoal differential agar (CCDA)

Figure 1. Different agar plates from Oxoid for the detection of enteropathogens

1. Isolated total genomic DNA from fresh stool samples — "VIASURE RNA-DNA Extraction Kit" (CerTest Biotec, S.L.)

2. Real Time PCR on the thermocycler AriaMx

Three monoplex assays (VIASURE assay)

- "VIASURE *Campylobacter* Real Time PCR Detection Kit" (*invA* gene)
- "VIASURE *Salmonella* Real Time PCR Detection Kit" (16S rRNA gene)
- "VIASURE *Yersinia enterocolitica* Real Time PCR Detection Kit" (ail gene)

One multiplex assay (R-biopharm assay)

- "RIDA® GENE Bacterial Stool Panel" (*ttr* gene, 16rRNA gene and *ail* gene)

Figure 2.Scheme of comparison of VIASURE and R-biopharm assays for the detection of Salmonella, Campylobacter and Yersinia enterocolitica

Results

14/200 (7%) samples were positive for *Salmonella*, **40/200** (20%) for *Campylobacter* and **2/200** (1%) for *Yersinia enterocolitica* by **VIASURE Real-Time PCR assay**. **13/200** (6,5%), **33/200** (16,5%) and **2/200** (1%), respectively, by **R-biopharm assay** and **14/200** (7%. 7 *Salmonella* Typhimurium, 5 *Salmonella* Enteritidis, 1 *Salmonella* serogroup C1 and 1 *Salmonella* Paratyphi A), **27/200** (13,5%. 22 *C. jejuni*, 3 *C.coli* and 2 *Campylobacter spp*), **2/200** (1%. 2 *Y. enterocolitica* O:3) by **culturing.**

We found 7 false negative for *Campylobacter* and 1 false negative for *Salmonella* by R-biopharm assay and 13 false negative for *Campylobacter* by culturing (see ***Table 1***).

		Campylobacter			Salmonella			Yersinia enterocolitica		
qPCR VIASURE	qPCR R-biopharm	Culture method			Culture method			Culture method		
		+	-	Total	+	-	Total	+	-	Total
+	+	27	6*	33	13	0	13	2	0	2
+	-	0	7*	7	1	0	1	0	0	0
-	+	0	0	0	0	0	0	0	0	0
-	-	0	160	160	0	186	186	0	198	198
	Total	27	178	200	14	186	200	2	198	200

Discrepant samples for Campylobacter were tested by a third Real Time PCR assay, whose results show a total agreement with VIASURE assay.

Table 1. Prospective study results according to the "Statistical guidance on reporting results from studies evaluating diagnostic tests" from the Food and Drug Administration.

Fig 3. Example of several positive stool samples for Campylobacter (blue), Salmonella (green) and Yersinia enterocolitica (red) by VIASURE essay run on the thermocycler AriaMx. Values of Cq: sample nº1 (Cq 16,66); 2 (22,55); 3 (27,21); 4 (30,88); 5 (22,46). Threshold baseline fluorescence: 500

Fig 4. Example of several positive stool samples for *Campylobacter* (blue), *Salmonella* (green) and *Yersinia enterocolitica* (red) by R-biopharm essay run on the thermocycler AriaMx. Values of Cq: sample nº1 (Cq 16,94); 2 (23,40); 3 (29,21); 4 (32,85); 5 (24,65). Threshold baseline fluorescence: 500

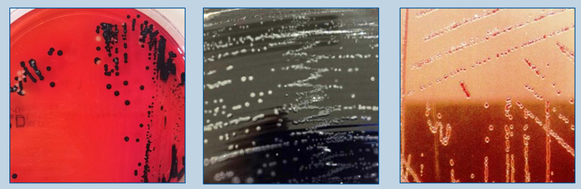

Fig 5. Colonies plated out from stool specimens.
A. Salmonella on XLD agar.
B. Campylobacter jejuni on CCDA.
C. Yersinia enterocolitica on CIN agar

Conclusions

1- Culture method can be considered a reliable technique to detect *Salmonella* and *Yersinia enterocolitica.* We found a total agreement between VIASURE Real-Time PCR assay and culture method for these pathogens.

2- Culture is less sensitive to detect *Campylobacter*, maybe because of the specific culture conditions required, which are different according to *Campylobacter* species. Some false negative obtained by culture method belong to patients which are in treatment.

3- Results show that molecular methods may constitute a faster, sensitive and specific diagnostic for the detection of these enteropathogens, being VIASURE Real-Time PCR assay the most sensitive one.

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