

Development and Validation of a Real-Time PCR Assay for Detection of *Yersinia enterocolitica* as Part of a Dutch Study on the Epidemiology of Gastro-Enteritis.

M.C. Scholts¹, G.J. Wisselink¹, R.F. de Boer¹, H.M.E. Maas², A.M.D. Kooistra-Smid¹, and Y.T.H.P. van Duynhoven³.

¹ Laboratory for Infectious Diseases, Department of Research & Development, Groningen, The Netherlands.

² National Institute of Public Health and the Environment, Centre for Infectious Disease Control, Department of Laboratory for Infectious Diseases and Screening, Bilthoven, The Netherlands.

³ National Institute of Public Health and the Environment, Centre for Infectious Disease Control, Bilthoven, The Netherlands

Introduction

Yersinia enterocolitica is an important human enteroinvasive pathogen with global distribution. Most commonly, yersiniosis is associated with self-limiting gastroenteritis. However, rapid diagnosis of yersiniosis is important, since it may result in early treatment of secondary, post infectious sequelae, like acute and chronic arthritis.

The gold standard for the detection of *Y. enterocolitica* in stool samples is a selective overnight culture followed by biochemical testing, which is a laborious and time-consuming method.

In May 2008, a study in 6 Dutch hospitals will commence to assess the incidence, etiology and course of patients hospitalized for gastro-enteritis (GEops study). As part of this study, and to facilitate rapid diagnosis, a real-time PCR assay was developed and validated for the detection of *Y. enterocolitica*.

Materials and Methods

Among pathogenic *Y. enterocolitica* strains there are 6 biotypes and several different serotypes. Strains of biotype 1A possess the *ystB* gene, encoding a heat stable toxin. Biotypes 1B, 2, 3, 4 and 5 possess the *ail* and *ystA* genes, encoding the attachment invasion locus and a heat stable toxin respectively. To detect all *Y. enterocolitica* biotypes, real-time PCR assays targeting the *ail*, *ystA* and *ystB* genes were developed.

DNA isolation from samples was performed with the easyMAG specific A stool protocol (bioMérieux). As internal control, phocine herpes virus-1 was used.

The selectivity of the assay was validated with a panel of characterized *Y. enterocolitica* isolates, biotypes 1A, 1B, 2, 3, 4 and 5 (n=61), a panel of *Yersinia non-enterocolitica* isolates (n=31) and a panel of non-*Yersinia* strains (n=35). Analytical sensitivity was assessed by dilution series (n=2), spiked in two different fecal matrices, one of liquid consistency and one of fixed consistency.

Also, a clinical validation was performed on stool samples routinely screened for bacterial and parasitic enteric pathogens (n=188).

Results

The assays proved to be specific for *Y. enterocolitica*, as no cross reaction was observed. All forty isolates of biotypes 1B, 2, 3, 4 and 5, scored positive in the real-time PCR for the targets *ail* and *ystA*. Of the 21 biotype 1A isolates, 20 scored positive for the *ystB* target. One biotype 1A isolate scored positive for the *ail* target and negative for the *ystB* target (Table 1). Sequencing confirmed this strain to be a *Y. enterocolitica*.

Table 1 Inclusivity of characterized *Y. enterocolitica* strains

Biotypes	<i>ail</i>	<i>ystA</i>	<i>ystB</i>
Concordant results			
1A (n=20)			+ (n=20)
1B (n=2)	+ (n=2)	+ (n=2)	
2 (n=1)	+ (n=1)	+ (n=1)	
3 (n=20)	+ (n=20)	+ (n=20)	
4 (n=15)	+ (n=15)	+ (n=15)	
5 (n=2)	+ (n=2)	+ (n=2)	
Discrepant results			
1A (n=1)	+ (n=1)		

Results continued

The *ystA* and *ail* assays were capable of detecting approximately 4400 CFU per gram of stool (for both fecal matrices). The *ystB* assay was capable of detecting approximately 3520 CFU per gram of stool (for both fecal matrices).

The assays possess a good linearity in a fecal matrix over a range of 5 log with a slope between 3.084 – 3.2707 and $r^2 > 0.977$. PCR efficiencies are 100%, 110% and 110% for *ail*, *ystA* and *ystB* respectively for both fecal matrices. Figure 1 shows the mean linearity per target in feces.

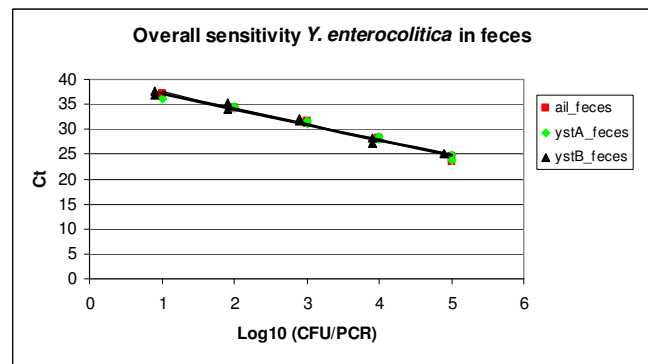


Figure 1 Overall sensitivity *Yersinia enterocolitica* in feces.

Y. enterocolitica DNA was not detected with these real-time PCR assays in any of the 188 clinical samples. PCR inhibition was observed in less than 1% of these samples. (Table 2)

Table 2 Clinical validation of stool samples screened for *Y. enterocolitica*

PCR	+	0
	-	187
	Inhibition	1
Total		188

Conclusions

- A real-time PCR assay targeting *ail*, *ystA* and *ystB* proved to be a sensitive and specific method for detection of *Y. enterocolitica*.
- Screening on both *ail* and *ystA* for biotypes 1B, 2, 3, 4 and 5 did not provide any additional results. Screening these biotypes with both target genes is not relevant. So, *ail* and *ystB* will be used as screening targets for detection of *Y. enterocolitica* in human stool samples.
- This real-time PCR assay will be used as a rapid screenings tool in the GEops study.