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26th ECCMID

European Congress of Clinical Microbiology and Infectious Diseases

Amsterdam, Apr. 9th to 12th

1. Comparison of two commercial Real Time PCR assays for detection of parasitic infections

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

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Comparison of two commercial Real Time PCR assays for detection of parasitic infections

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Background

Enteric protozoa continue to be the most commonly encountered parasitic diseases affecting millions of people each year and causing significant morbidity and mortality worldwide. Among them, **Cryptosporidium** spp., *Entamoeba histolytica*, and *Giardia lamblia* are the major etiological agents.

Microscopy has been considered to be the gold standard method for diagnosis of these parasites, however is time-consuming, not very sensitive and unable to distinguish the invasive pathogenic *E. histolytica* from the commensal parasite *E. dispar*, which is 10 times more common worldwide. Real Time PCR assay is less labor-intensive and has higher sensitivity and specificity, making it an attractive alternative. Novel ready and easy-to-use assays as "**VIASURE Real Time Detection kits**" offers the routine lab additional advantages due to not require trained personnel and minimizes the number of manipulations reducing time and possible errors.

The aim of this study is to compare two different commercial Real Time PCR assays targeting 18S rRNA gene for the specific detection of *Cryptosporidium*, *Entamoeba histolytica, Entamoeba dispar* and *Giardia lamblia* DNA in stool samples.

Material / Methods

A prospective comparative study was carried out in 150 faecal samples from patients with clinical suspicion of parasite diseases collected from September 2014 and August 2015. Nucleic acid extractions were performed using **"Viasure RNA-DNA Extraction kit"** (Certest Biotec).

Samples were analyzed using four monoplex assays in parallel since share same thermal cycling protocol: "**VIASURE *Cryptosporidium* Real Time PCR Detection Kit**", **"VIASURE *Entamoeba histolytica* Real Time PCR Detection Kit"**, **"VIASURE *Entamoeba dispar* Real Time PCR Detection Kit"** and **"VIASURE *Giardia lamblia* Real Time PCR Detection Kit"** (Certest Biotec). Besides they were evaluated with a commercial multiplex assay "RIDA®GENE Parasitic Stool Panel II" (R-Biopharm). Any discrepant results were tested with an in-house Real Time PCR assay.

Results

A total of 104 cases of 150 were diagnosed (70%) (2 *Entamoeba histolytica*, 21 *Entamoeba dispar*, 38 *Cryptosporidium* and 48 *Giardia lamblia*) using **VIASURE** Kits. Co-infections of *Cryptosporidium* and *Giardia lamblia* were identified in 5 positive specimens. However, only 86/150 samples were positive for R-Biopharm (2 *Entamoeba histolytica*, 36 *Cryptosporidium* and 48 *Giardia lamblia*).

Agreement between **VIASURE** and R-biopharm for the detection of *Entamoeba histolytica* and *Giardia lamblia* was 100%; whereas we found a concordance for *Cryptosporidium* of 90%, with 3 samples found to be only positive with **VIASURE** and 1 case in R-biopharm. All these discrepant cases were evaluated by an in-house assay reported by Hadfield et al., 2011, confirming our results.

Conclusions

Although the performance of **VIASURE** and R-biopharm assays were overall comparable, **VIASURE** assays showed better sensitivity and specificity for the detection of *Cryptosporidium*. Besides, **VIASURE** allowed detection and differentiation of pathogenic invasive amoeba *E. histolytica* from *E. dispar*, which is crucial to the clinical management of patients.

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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

We performed a comparative prospective study at Hospital Clínico Universitario Lozano Blesa (Spain), where we tested 200 stool samples (October 2015-November 2015) from patients with gastrointestinal symptoms.

Total genomic DNA was isolated from fresh stool samples with the commercial Kit **"VIASURE RNA-DNA Extraction Kit"** (Certest Biotec S.L). Nucleic acids were amplified on thermocycler AriaMx (Agilent Technologies) using three monoplex assays **"VIASURE *Salmonella* Real Time PCR Detection Kit"** (invA gene), **"VIASURE *Campylobacter* Real Time PCR Detection Kit"** (16S rRNA gene) and **"VIASURE *Yersinia enterocolitica* Real Time PCR Detection Kit"** (ail gene) (Certest Biotec S.L) in comparison to the multiplex assay ""RIDA®GENE Bacterial Stool Panel" (R-biopharm). Discrepant samples were tested by a third Real Time PCR assay "mericon Campylobacter spp Kit" (Qiagen ®).

Relative to culture method, all samples were cultivated in six different culture medium: selenito broth, xylose lysine deoxycholate agar, MacConkey agar, Hektoen enteric agar, Cefsulodina-Irgasan-Novobiocina agar and *Campylobacter* charcoal differential agar (Oxoid).

Results

(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. Results of discrepant samples obtained by the third Real Time PCR assay support all results obtained by **VIASURE** assay.

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.