Evaluation of the Fast-track Diagnostics® (FTD) Bacterial gastroenteritis real-time multiplex PCR for the detection of the main pathogens in stool samples

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Introduction
Bacterial gastroenteritis is a common clinical problem. The diagnosis relies mainly on stool culture and takes several days. The only rapid and reliable test currently used is for *Clostridium difficile*. We evaluated the multiplex PCR panel of Fast-track Diagnostics® for the detection of the main bacterial gastroenteritis pathogens by comparing it to standard stool culture and established antigen detection and PCR methods.

Objective
The aim of this study was to evaluate a multiplex PCR for the detection in faeces of the main bacterial pathogens responsible for gastrointestinal infections: *Salmonella* sp, *Shigella* sp, *Campylobacter* sp (jejuni and coli), *Clostridium difficile*, *Yersinia enterocolitica*. Vero cytotoxin producing and enteroinvasive *E.Coli* were not included in the evaluation. We also evaluated the advantages and disadvantages of such a method for a microbiology laboratory.

Material and methods

Samples (n=86)
- 63 positives stool samples
  - 36 native stools standard positive for:
    - *Campylobacter* coli (14)
    - *Salmonella* serovars (13)
    - *C difficile* (10)
  - 25 stools spiking with 10^9 bacterial cells/mL
    - *Salmonella* sp (15) including 20 serovars, *C difficile* (5)
    - *Shigella* (2) including 2 serovars, *Yersinia* enterocolitica (5)
- 23 negative stool samples
  - 11 native stools without detectable pathogen
  - 11 stools spiked with 10^9 bacterial cells/mL
  - Vibrio *sp* (9)
  - *Yersinia enterocolitica* (2)
- 1 stool toxin-negative *C difficile

Kit Fast-track Diagnostics® Bacterial gastroenteritis
Two tube multiplex PCR for detection of:
- *Salmonella* sp, *Campylobacter* coli/jejuni, *Clostridium difficile*, *Yersinia enterocolitica* VTED and internal control by TaqMan® technology.

Real time PCR
The stool samples were resuspended 1:10 in PBS and frozen at -20°C before extraction on the QIASymphony®. The assay setup was performed manually due to the low reagent volume and dead-volume of the assay setup module. The PCR conditions were according to the instructions of the manufacturer.

Results

Sensitivity and specificity

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<tr>
<td>100</td>
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<td>86</td>
<td>87</td>
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Sensitivity is between 86 et 100 %
Specificity 100%

Time and costs

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| The detection time for the PCR is 4 hours. The turn-around time for the culture is about 24-48 hours, except for *C difficile* for which the reference tests are the Techlab C.diff Quik Chek Complete® (results in 30 minutes) and PCR for *C difficile* toxins (Cepheid GenoXpert®).

Cost of the analysis

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<th>Culture</th>
<th>PCR</th>
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<td>24</td>
<td>34</td>
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<td>85</td>
<td>110</td>
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Discordant results

*Campylobacter* jejuni
Two Campylobacter jejuni culture positive samples were negative with the FTD® kit. Since the internal control was also negative, a PCR inhibition is probable.

*Clostridium difficile*
Two C.difficile samples were not detected but were positive with the C.diff Quik Chek Complete® and the GenoXpert® (CT values of 27.6 and 28.7).

For one of the samples, a PCR inhibition is probable (internal control negative). For the other discordant sample, a lower sensitivity of the FTD® kit, possibly due to the dilution of the stool before extraction on the QIASymphony, might be an explanation.

Conclusion

Fast-track Diagnostics® advantages
- Fast detection of 7 pathogens with good sensitivity and specificity.
- Easy samples preparation and use of the kit.

Fast-track Diagnostics® disadvantages
- Difficult to integrate in an automated system because of a lack of reagents (dead volume issue).
- Manual pipetting with risks of errors.
- More expensive than standard method.

Conclusion
The Fast-track Diagnostics® kit is rapid and reliable for the detection of bacterial pathogens in stools. However, this method cannot completely replace the culture because of the need of antibiotic susceptibility testing for positive samples. Once the method available in a fully automated system and less expensive, its place in the microbiology laboratory might be useful to rule out bacterial pathogens. Cultures could be limited to positive stool samples requiring antibiotic susceptibility tests.