R361
Publication Only
Diagnostics, other than Molecular: Diagnostic/laboratory methods (other than molecular)

Clostridium difficile diagnostics: comparative study of two immunoenzymatic assays with confirmation by real-time PCR and anaerobic cultivation with PCR ribotyping

M. Krutova[1], J. Matejkova[1], M. Zajac[1], P. Hubacek[1], O. Nyc[1]

[1]Medical Microbiology, Charles University 2nd Faculty of Medicine University Hospital Motol, Prague 5, Czech Republic

Objectives: The comparison of the sensitivity and specificity of two commercial immunoenzymatic assay for the detection of *C. difficile* GDH (glutamate dehydrogenase) and toxins A / B.

Material and Methods:

A total of 86 liquid/unformed stool samples from patients hospitalized at the University Hospital Motol were tested in a period from May to June 2013. GDH and toxin A / B tests were performed by *C. difficile* Quik Chek Complete®, (Alere, USA) and Liaison® *C. difficile* GDH and Toxins AαB (DiaSorin USA). Nucleic acids extractions were performed by UltraClean® fecal DNA, (MoBio Laboratories, USA) and further PCR amplification by Real-Time PCR kit *C. difficile* Elite® MGB (Nanogen, Italy). Anaerobic cultivation on selective medium for *C. difficile* (Oxoid) was performed in samples with a positivity at least one assay. PCR ribotyping based on capillary electrophoresis was performed according to the European *Clostridium difficile* infection surveillance network (ECDIS-net) Standard Operation Protocol.

Results:

36 (42%) samples were GDH and toxin A / B negative by the both of methods. 20 (23%) samples were GDH and toxin positive by the both of methods. 9 (10%) samples were GDH positive and toxin negative by the both of methods, but PCR positive. 11 (13%) samples were GDH positive and toxin negative by the both of methods, but PCR negative. 6 (7 %) of samples were GDH positive and toxin positive only by Liaison® test. 4 (5%) samples were GDH positive only by Liaison® test. The PCR reaction were inhibited in 5 cases (6%), the repeat of nucleic acid extraction were needed. These *C. difficile* toxigenic ribotypes were identified: AI -3, 001, 002, 012, 014, 017, 020, 049, 054, 078, 176, 203, 413. And *C. difficile* non-toxigenic ribotypes were: AI -34, AI- 61, 010, 485, 495, 596.

Conclusion:

The test Liason® *C. difficile* toxins A / B had about 7% higher sensitivity, than *C. difficile* Quik Chek Complete®. We believe that paramagnetic beads provide better surface for the bindings of antibody-antigen complex. Two-step test arrangement (no further testing of GDH negative samples) also brings economic savings that can be used to increase testing of CDI or to add PCR methods to the laboratory diagnostic algorithm of CDI.

Support:

Diagnostic kits were supplied by DiaSorin Czech, and Elisabeth Pharmacon Ltd. Cultivation and PCR Ribotyping was supported by grants: Ministry of Health - RVO, FN Motol 00064203 and MH CZ IGA NT/14209.