EVALUATION OF A CHROMOGENIC MEDIUM FOR THE ISOLATION OF CLOSTRIDIUM DIFFICILE FROM STOOL SAMPLES USING AN AUTOMATED INOCULATION SYSTEM.

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Introduction
Since the end of the seventies, Clostridium difficile has emerged as a major nosocomial pathogen. The main virulence factors are two high molecular weight exotoxins, namely toxins A and B that both exhibit cytotoxic and enterotoxicty.

Discussion
Manual inoculation is still superior, since the stool sample is not pre-treated. In Cary Blair medium, Chrom ID (bioMérieux) gave the best results. On Manual inoculation as well as on the BD Innova automated inoculation system, the method allows a major reduction of the incubation period (24h). Another point of attention is that, after 24h incubation, the toxigenic status of the strain must be determined by a molecular biology method instead of an immunoassay. All suspicious colonies were analysed with the MALDI-TOF MS (Bruker). Two C. difficile strains were not recognised in MALDI-TOF but could be recognised with a binocular. Some ribotypes (020,021,014) of C. difficile, stayed uncoloured but with the typical colony-shape for C. difficile.

Materials and methods
Stools were from infants (≥2y) suffering from antimicrobial or hematotherapy associated diarrhoea. 290 stools collected over a 14 weeks period (between Nov 2011 and Jan 2012) were tested.

Inoculations:
Manually using a 10 µL loop.

Automated inoculation with a 30 µL loop on BD®Innovia after dilution of about 50 μL in 2ml of Cary Blair

Cultures:
Cycloserine-Cetoxamin-Fructosa-egg-yolk-Agar CCFA, homemade (twice overnight anaerobic incubation at 35°C).

Chrom ID (bioMérieux, Lyon, France), (24 h anaerobic incubation at 35°C).

CCEY (Oxoid, Wesel,Germany), (twice overnight anaerobic incubation at 35°C).

CDSA (BD, Sparks MD USA), (twice overnight anaerobic incubation at 35°C).

Cary Blair medium (Copan,Brescia,It.) was used to perform automated inoculation.

Reading cultures:
All cultures were read with a binocular stereomicroscope, with the lightbeam through the Petri dishes under a certain angle.

Identification:
Mild ToF MS biotyper was used to identify the C. difficile colonies.

Sample preparation:
The picked swab of the Cary Blair medium, collects ~150mg of fecal material.
The screw-cap tube contains 2 ml of modified semi-liquid Cary Blair medium.
Samples are pretilted ~13 times.

Results

Table 1 Comparison stools inoculated manually and with BD®Innovia

<table>
<thead>
<tr>
<th>n</th>
<th>CCFA (manually)</th>
<th>CCFA (Innova)</th>
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</thead>
<tbody>
<tr>
<td>POS</td>
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<td>63</td>
</tr>
<tr>
<td>NEG</td>
<td>210</td>
<td>327</td>
</tr>
</tbody>
</table>

Table 2 Comparison different culture media inoculated with BD®Innovia

<table>
<thead>
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<th>n</th>
<th>CCFA (manually)</th>
<th>Chrom ID (manually)</th>
<th>Chrom ID (bioMérieux)</th>
</tr>
</thead>
<tbody>
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<td>POS</td>
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<td></td>
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<tr>
<td>NEG</td>
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Table 3 Comparison CCFA and Chrom ID manually

<table>
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<th>n</th>
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<th>Chrom ID (manually)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POS</td>
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<td>63</td>
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<tr>
<td>NEG</td>
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</table>

Fig.1a BD®Innovia

Fig.1b Different inoculations on BD®Innovia

Fig.2 Clostridium difficile on ChromID agar (bioMérieux)

Fig.3 Binocular stereomicroscope

References

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