Sterility testing using a rapid microbiological method for batch production of cytotoxic drugs in a hospital pharmacy

A. Matheron1, R. Vazquez2, M.N. Guerrault-Moro1, D. Brossard1, S. Crauste-Manciet1.

1CHI de Poissy / St Germain en Laye, Pharmacy, St Germain en Laye, France.

**Background**

- Implementation of batch production of standardized doses of 11 cytotoxics and 1 monoclonal antibody using Repeater® pump (Baxa, Baxter)
- Necessity of implementation of physicochemical and sterility tests for batch release according to French good manufacturing practice for hospital pharmacies
- Possible inhibition of microorganism growth with cytotoxics 2-4

**Purpose**

To investigate the possible use of rapid microbiological method (BD Bactec®) for sterility testing of the cytotoxic batches

**Materials and methods**

**First step**

Inoculation with <100 Colony-Forming-Unit (CFU) in cytotoxic bags

- 4 microorganisms recommended in European Pharmacopoeia
- 3 additional microorganisms from human microflora which can contaminate cleanroom

**Second step**

Detection of microbial growth of cytotoxic bags with Bactec® system

- (CO2 detection by fluorescence)

**Third step**

- When no microbial growth:
  - investigation of 1/10 and 1/100 dilution of initial solution
  - use of another culture media of rapid microbial analysis BactAlert®, Biomerieux

**Results**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Automate</th>
<th>C⁰ (mg/ml)</th>
<th>SA</th>
<th>PA</th>
<th>BS</th>
<th>CA</th>
<th>SE</th>
<th>EC</th>
<th>EF</th>
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</thead>
<tbody>
<tr>
<td>5-Fluourouracil (5FU)</td>
<td>Bactec®</td>
<td>22</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>Bactec®</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>Bactec®</td>
<td>2.2</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>Bactec®</td>
<td>0.2</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Oxaliplatin</td>
<td>Bactec®</td>
<td>0.5</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>Epirubicin</td>
<td>Bactec®</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Bactec®</td>
<td>4</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>Docetaxel</td>
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<td>+</td>
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<td>Paclitaxel</td>
<td>Bactec®</td>
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<td>+</td>
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<tr>
<td>Etoposide phosphate</td>
<td>Bactec®</td>
<td>1.15</td>
<td>-</td>
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<td>Irinotecan</td>
<td>Bactec®</td>
<td>2.25</td>
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<td>+</td>
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</tr>
</tbody>
</table>

**Discussion**

Combination of sterility tests with Bacterial Endotoxin Test 6 7 would contribute to improve the results for gram-negative bacteria.

**Conclusion**

This study shows the interest of validation of microbial method for implementation of sterility test when using drugs to exclude a risk of false negative results.

1AFSSAPS, Good manufacturing practices, November 2007.
5European Pharmacopoeia 7.2 - Biological methods - Chapter 2.6.1 Sterility. Edigm.
6United States Pharmacopeia, General Chapter <66> Bacterial Endotoxins Test. United States Pharmacopoeia Convention; Rockville, MD.

For most of the cytotoxic drugs, microbial growth was observed with the 7 microorganisms investigated excepted for 5FU and gemcitabine.

1/100 dilution or the use of BactAlert® on concentrated solution of 5FU allowed recovering growth of SA, SE, CA but only CA for gemcitabine where Staphylococcus species were not able to grow whatever the investigated conditions.

5FU and gemcitabine inhibited the growth of EF and BS whatever the culture media and the dilution (1/10, 1/100).

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