

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



A. Matheron¹, R. Vazquez¹, M.N. Guerrault-Moro¹, D. Brossard¹, S. Crauste-Manciet¹.

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

TCH-044

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 pharmacies¹
- Possible inhibition of microorganism growth with cytotoxics²⁻⁴



Purpose

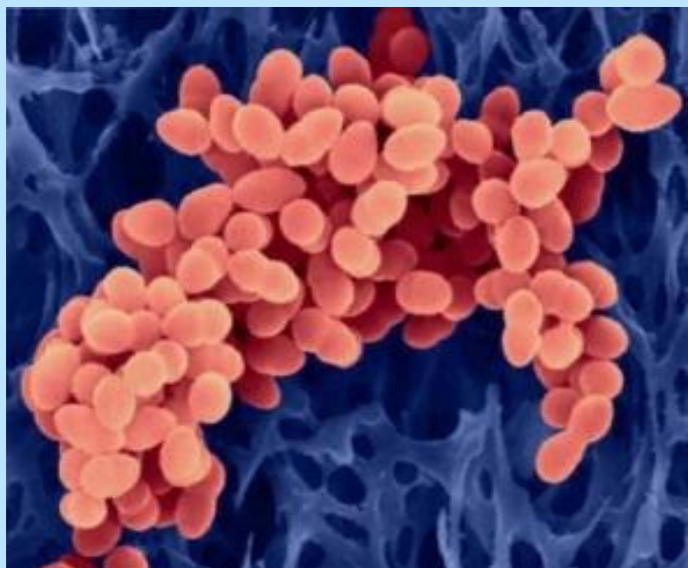
To investigate the possible use of **rapid microbiological method (BD Bactec[®])** for sterility testing of the cytotoxic batches

Materials and methods

First step

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

4 microorganisms recommended in European Pharmacopeia⁵



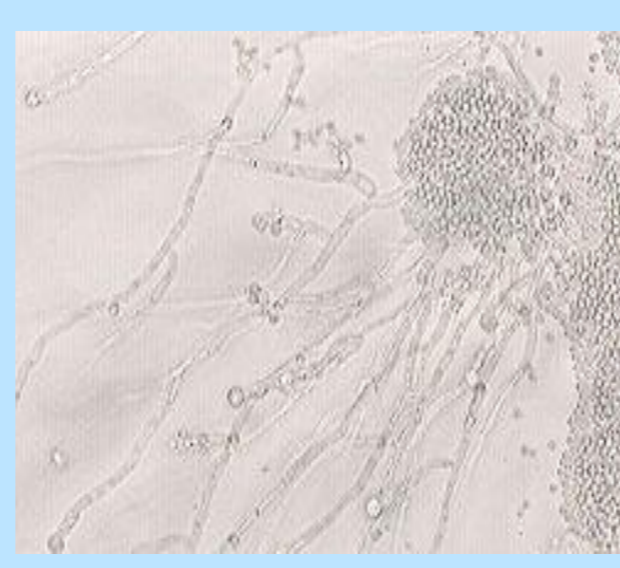
Staphylococcus aureus (SA)



Pseudomonas aeruginosa (PA)



Bacillus subtilis (BS)



Candida albicans (CA)



Staphylococcus epidermidis (SE)



Escherichia coli (EC)

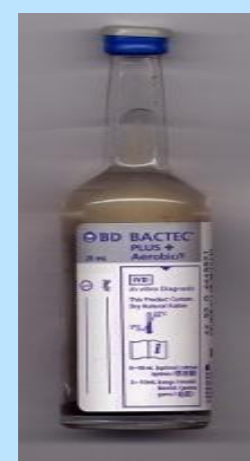


Enterococcus Faecalis (EF)

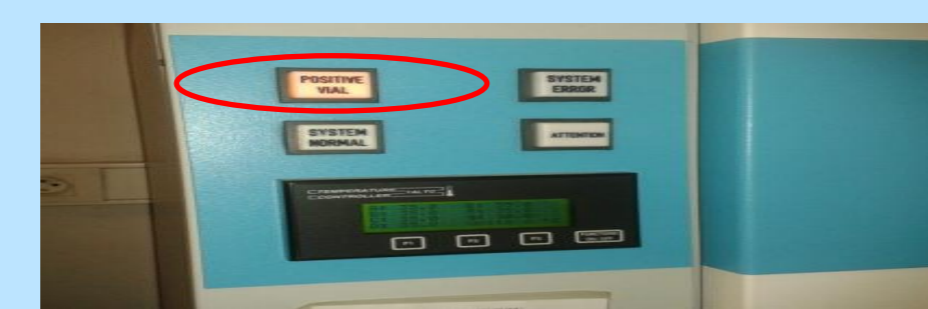
3 additional microorganisms from human microflora which can contaminate cleanroom

Second step

Detection of microbial growth of cytotoxic bags with Bactec[®] system (CO₂ detection by fluorescence)



Aerobic or anaerobic Bactec[®], BD



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

Drug	Automate	C° (mg/ml)	SA	PA	BS	CA	SE	EC	EF
5-Fluorouracil (5FU)	Bactec [®]	22	-	-	-	-	-	-	-
Gemcitabine	Bactec [®]	10	-	+	-	+	-	-	-
Carboplatin	Bactec [®]	2	+	+	+	+	+	-	+
Cisplatin	Bactec [®]	0.2	+	-	-	+	+	-	+
Oxaliplatin	Bactec [®]	0.5	+	-	+	+	+	+	+
Epirubicin	Bactec [®]	2	-	-	-	+	-	+	+
Cyclophosphamide	Bactec [®]	4	+	+	+	+	+	+	+
Docetaxel	Bactec [®]	0.68	+	-	-	+	+	+	+
Paclitaxel	Bactec [®]	0.6	+	-	-	+	+	+	+
Etoposide phosphate	Bactec [®]	1	+	-	+	+	+	+	+
Irinotecan	Bactec [®]	1.15	+	-	+	+	+	+	+
Trastuzumab	Bactec [®]	2.25	+	+	+	+	+	+	+

+

Detection of microorganism in Bactec[®]



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Absence of detection of microorganism in Bactec[®]



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.

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).

¹AFSSAPS, Good manufacturing practices. November 2007.

²Krämer I. Viability of microorganisms in novel antineoplastic and antiviral drug solutions. *J Oncol Pharm Practice*. 1998; 4 (1): 32-37.

³Paris I, Paci A, et al. Microbial growth tests in anti-neoplastic injectable solutions. *J Oncol Pharm Practice*. 2005; 11: 7-12.

⁴Rawal BD. Variation in microbial survival and growth in intravenous fluids. *Chemotherapy*. 1985; 31(4): 318-323.

⁵European Pharmacopeia 7.2 - Biological methods - Chapter 2.6.1 Sterility. Edqm.

⁶United States Pharmacopeia, General Chapter <85> Bacterial Endotoxins Test. United States Pharmacopeial Convention: Rockville, MD.

⁷Guidance for Industry - Pyrogen and Endotoxins Testing: Questions and Answers on <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM310098.pdf> consulted october 10th 2012.