STERILITY CONTROL OF AQUEOUS AND OILY EYE DROPS USING BACTEC® AUTOMATED SYSTEM: FEASIBILITY AND VALIDATION

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INTRODUCTION

The Central Pharmacy of the Mayotte Hospital Centre produces hospital preparations of eye drops based on aqueous or oily excipients. These eye drops are used immediately after manufacture on the basis of parametric release, as the sterility test prescribed by the European Pharmacopoeia requires up to 14 days of incubation. The aim of this study is to validate a rapid technique for the sterility checking of eye drops preparations using Bactec® technology based on an early detection of CO_2 production (DCP) with color change in case of microbes growth.

MATERIEL AND METHODS

- Two series of eye drop preparations (9.9 mL/bottle, n=6/preparation) were made under aseptic conditions, in a laminar flow hood, without adding active ingredients: aqueous eye drops (AED) based on 0.9% NaCl and oily eye drops (OED) based on castor oil. The experiments was repeated 3 times.
- Six ATCC reference microorganisms recommended by the European Pharmacopeia were used (Bioball®, 550 CFU/unit): *Staphylococcus aureus* (G1), *Pseudomonas aeruginosa* (G2), *Clostridium sporogenes* (G3), *Bacillus subtilis* (G4), *Aspergillus brasiliensis* (G5) and *Candida albicans* (G6)
- These microorganisms were resuspended in 0.9% NaCl solution, were used for the validation studies. The preparations were enriched with 100µL of each reference germ solution (final concentration 1CFU/mL), then mixed for during 1 min.
- Aliquots of 3 mL of eye drops (3 CFU) were introduced into three different DCP media: BACTEC Peds (B1, aerobic medium), BACTEC™ Plus Anaerobic (B2) and BACTEC Plastic Mycosis IC/F (B3, filamentous fungi), then incubated in a BD Bactec Fx40 incubator (Figures 1 and 2).
- Identical aliquots were cultured in the media prescribed by the European Pharmacopeia: Tryptone Soy Broth (TSB) and Thioglycolate Broth (TB). For the COE, 3mL aliquots were filtered through a 0.22µm membrane filter (FM) and then transferred from the filter to Count-tact® agar.
- Positive and negative controls were used in each series of experiments.
- Two parameters were evaluated: detection or non-detection of the germ (readings taken over a minimum of 5 days) and time to obtain a positive result (Time to Detection or TTD).

RESULTS AND DISCUSSION

- The B1 (Bactec PEDs) and B3 (Bactec Plastic Mycosis) media detected all germs except G3, an anaerobic bacteria.
- G3 (*Clostridium sporogenes*, anaerobic) was difficult to detect on conventional media and by membrane filtration and required incubation in an anaerobic generator. However, it was easily and quickly detected with the B2 medium specific to anaerobes.

		G1 *S. aureus*	G2 *P. aeruginosa*	G3 *C. sporogenes*	G4 *B. subtilis*	G5 *A. brasiliensis*	G6 *C. albicans*
BACTEC Peds	Aqueous	**8h41**	**09h35**	Negative	**7h58**	21h56	12h31
	Oily	**9h17**	**10h55**	Negative	**9h05**	22h31	16h21
BACTEC™ Plus Anaerobic	Aqueous	8h47	10h32	**17h16**	Negative	17h38	9h45
	Oily	9h49	12h03	**18h21**	Negative	19h35	13h45
BACTEC Plastic Mycosis IC/F	Aqueous	15h14	12h32	Negative	**7h51**	**7h02**	**7h19**
	Oily	18h21	12h48	Negative	**8h31**	**7h48**	**7h49**

Table 1: Average Time to Detection (n=3) for each microorganism according to the nature of the matrix and the culture medium used *(standard errors not shown)*

Figure 1: Bactec® FX 40 incubator

Figure 2: Media B1, B2 and B3 media used

- Media B1 and B3 detected all germs in different matrixes, with varying detection speeds, except for anaerobic microorganisms.
- For our routine operations, we have decided to use only B1 medium to detect aerobic germs and filamentous fungi with both aqueous and oily matrixes. We will use medium B2 to detect anaerobic bacteria.
- Detection of all germs was faster with the DCP technique, in all cases <24 hours regardless of the matrix, with detection times ranging from 7h02±18min for Aspergillus in aqueous media to 18h21±29min for Clostridium in oily media (Table 1).
- With the reference techniques of the European Pharmacopoeia, detection required between 3 and 5 days of incubation to obtain a positive result.

CONCLUSION

The DCP technique enabled rapid detection of reference microorganisms present in aqueous or oily eye drops, with a low detection limit (1CFU/mL) and a low total number of incubated CFUs (3 CFU).
The performance of the technique was better than the reference techniques in the European Pharmacopoeia: rapid (<24 hours), repeatable, sensitive and covering all the bacteria used, which will allow hospital eye drop preparations to be released within 24 hours.