APPLICATION NOTE 90550

Verification of the “ContraCon decontamination routine” with the Cytomat 10 C Incubator

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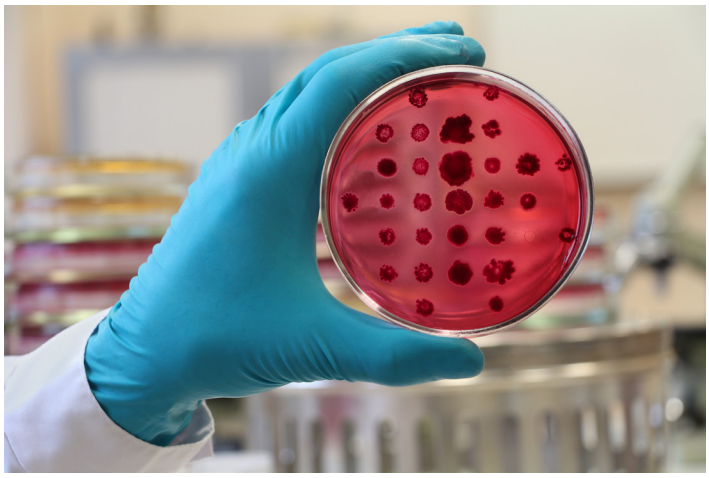


Goal

To verify the ContraCon™ decontamination routine (9 hours, 90 °C, moist heat >80% relative humidity) at defined positions within the Thermo Scientific™ Cytomat™ 10 automated incubator.

Introduction

Cell cultures are used for basic biomedical and pre-clinical research as well as for pharmaceutical bio production. Even though aseptic cell culture practices are well known, contamination is still an issue that can occur through inadequately aseptic handling or the use of contaminated media or inoculant. Microbiological contamination in cell cultures can be caused by bacteria, fungi and viruses, and can lead to a tremendous loss of product or experimental data points. Mitigating contamination risk is especially important for devices, such as incubators, where microbes encounter optimal growth conditions. As a result, a proper decontamination routine is essential for such devices. Automated systems with integrated handling components and sensors, need a special strategy to prevent contamination and keep the technical equipment sterile.

The current study validated that the ContraCon decontamination routine for the Cytomat 10 C incubator that was developed to prevent and decontaminate this automated system. The Cytomat 10 C incubator integrates into automated workflows and is equipped with a plate handling system, barcode reader and a carousel with racks that offer space for up to 210-well plates.

Equipment

- Cytomat 10 C automated incubator
- Thermo Scientific™ Heracell™ CO_2 incubator
- Cytation™ 5 cell imaging reader, BioTek®
- GloMax® Discover Microplate Reader, Promega™

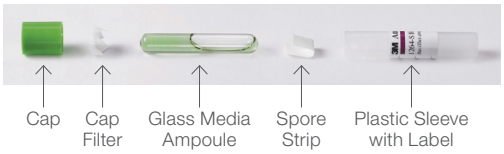
Reagents and standards

B.subtilis / B.atrophaeus [ATCC® 9372™] and *Escherichia coli* [ATCC® 9637™]

Procedure

To test decontamination, the Cytomat 10 C was equipped with test tubes containing different microorganisms. *B.subtilis / B.atrophaeus* [ATCC® 9372™] and *Escherichia coli* [ATCC® 9637™] serve as reference test organisms for common cell culture contaminants. The test tubes were placed on 18 different positions in the incubator that included walls (bottom, top, sides) and places behind/ shielded by technical equipment. The ContraCon (9 hours, 90 °C, moist heat >80% rel. humidity) decontamination routine was performed. The test tubes were subsequently removed and further incubated to analyze residual growth of the microorganisms. Control test tubes which were not subjected to the decontamination treatment were incubated in parallel.

Table 1. Test organisms and test mode

Organism	Test mode
B.subtilis / B.atrophaeus [ATCC® 9372™]	bio indicator, post-incubation 48 hours, yellow color indicates bacterial growth 
Escherichia coli [ATCC® 9637™]	test tube with suspension of OD 0.8, post-incubation: • aliquot 1: 37 °C / Caso-broth, OD-measurement 2 hours / 24 hours; • aliquot 2: plated on LB-agar, colony count 24 hours / 7 days

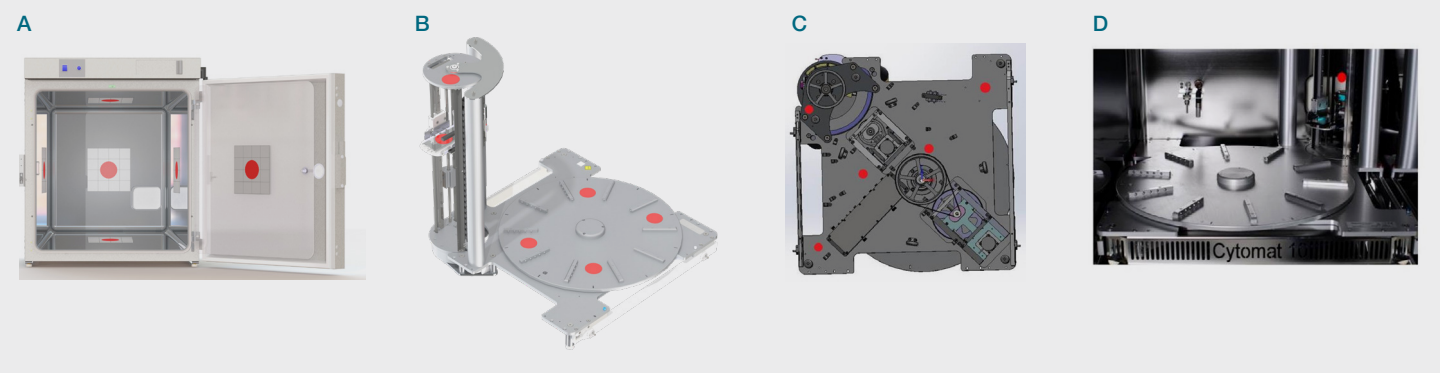


Figure 1. Positions of test tubes in the Cytomat 10 C incubator during ContraCon. A) Interior walls of the incubator housing with depicted test positions. B) Test positions on the bottom of the rotating plate, on top of plate shuttle system and on top of the plate shuttle rack. C) Test positions below rotation plate D) Test positions near the back gate.

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Results

B. atrophaeus

Table 2. Test results for *B. atropheus*. Bacterial growth after 48 hr post-incubation test tubes on specific positions in the incubator during the ContraCon decontamination routine.

	Position	Bacterial growth within 48 hours post-incubation
1	Interior walls (right, left, top, bottom wall and glass door) (6 positions)	–
2	Bottom / below rotating plate (5 positions)	–
3	On top of the rotating plate (4 positions)	–
4	On top of the plate shuttle	–
5	On top of the plate shuttle rack	–
6	Near the back door	–
7	Outside the incubator (without decontamination routine)	Yellow color = *B.atrophaeus* is active; processed incubator results are valid
8	HeraCell ContraCon, bottom and top shelf (2 positions)	–

Escherichia coli

Table 3. Test results for *E. coli*. Results of OD measurement after 24 hr and colony count after 24 hr and 7 days of sample on specific positions in the incubator during the ContraCon decontamination routine.

	Position	Bacterial growth Δ OD 24 hr	Colony count 24 hr / 7 days
1	Interior walls (right, left, top, bottom wall and glass door) (6 positions)	–	–
2	Bottom / below rotating plate (1 positions)	–	–
3	On top of the rotating plate (1 positions)	–	–
4	On top of the plate shuttle	–	–
5	On top of the plate shuttle rack	–	–
6	Near the back door	–	–
7	Outside the incubator (without decontamination routine)	+	Bacteria lawn
8	HeraCell ContraCon, bottom and top shelf (2 positions)	–	–

Conclusion

The results demonstrate the successful performance of the ContraCon decontamination routine for the Cytomat 10 C incubator against the two tested microorganisms. The procedure sufficiently eradicated contaminants even on the difficult to access places behind technical equipment in the incubator. Therefore, the Cytomat 10 C incubator is a system that integrates a safe decontamination routine and is easily applicable for automated cell culture processes.

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