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Beta Test: Real-time PCR-based Presence/ Absence Test for the Detection of Bacterial Contamination in ATMPs

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Abstract

In this study the robustness of the Microsart® ATMP Bacteria real-time PCR kit was demonstrated by spiking DMEM + 5 % FBS with *Clostridium sporogenes* (ATCC 19404) at 2x LOD_{95}, LOD_{95} and ½ LOD_{95} and tested by five participants, representatives of the biopharmaceutical industry and hospital associated labs. The spiked samples were shipped frozen to all participants for running tests with the Microsart® Bacteria Extraction and Microsart® ATMP Bacteria at their site. *Clostridium sporogenes* has been selected for this study because this is one of the worst case organisms for the test system, showing highest limit of detection.

The results of this study demonstrate that rapid real-time PCR-based detection of bacterial contamination is a robust and suitable method which could contribute to a risk reduction and thus to patient safety.

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Introduction

Sterility is a critical quality attribute of cellular therapeutics. Since microbial contamination of cell therapy products can potentially lead to life-threating infections of recipients sterility testing is a critical component of the release testing for any cell therapy product. The current compendial sterility test takes 14 days before a contamination can be ruled out with certainty which is too long for short shelf life cellular therapeutics, especially for autologous cell therapies intended to treat terminally ill patients. As a result there is an increasing demand for growth-independent rapid assays. Therefore, a detection system consisting of the highly efficient DNA extraction protocol of Microsart® Bacteria Extraction followed by the real-time PCR assay Microsart® ATMP Bacteria has been developed. A validation study was designed to evaluate the bacterial detection capability. The study was setup to meet requirements of the European Pharmacopoeia chapter 5.1.6 [1]. Additionally in silico sequence alignment analysis demonstrated that Microsart® ATMP Bacteria is able to detect > 94 % of Gram positive and Gram negative bacteria. This feature enables the detection of any bacterial contamination, even including species difficult to detect with the classical culture method.

In this study the robustness of the Microsart® ATMP Bacteria real-time PCR kit with prior DNA extraction was demonstrated with the help of a beta test. Spiked samples were successfully tested by five participants.

Materials and Methods

Each of the beta test participants received the required kits and consumables (Table 1). The required lab instruments and equipment, e.g. real-time PCR cyclers, have been made available by the participants. Spiked samples (Table 2) that were unknown to the participants were provided by Sartorius.

Table 1

Kits and consumables for the best test

Quantity	Product	Reactions
1	Microsart® ATMP Bacteria	100
1	Microsart® Bacteria Extraction	50
3	Biosphere® Filter Tips 1000 µl	-
2	Biosphere® Filter Tips 100 µl	-
2	Biopshere® Filter Tips 20 µl	-
2	PCR tubes á 12x 8-strip, High Profile	-
2	PCR tubes á 12x 8-strip, Low Profile	-
1	PCR Clean	-
1	PCR Clean Wipes	-
10	Pairs of Sleeve protectors	-
10	Hairnet	-
10	Mask	-

Table 2:

Unknown spiked samples for the beta test

Quantity	Volume per tube	Component	Label	Storage Temp.
1 tube	5 ml	Negative Extraction Control	NEC	2-8 °C
1 tube	15 ml	Matrix	Matrix	2-8 °C
8 tubes	1 ml	Unknown samples	1-8	-20 °C

All beta test participants followed the steps below:

1. 1 ml of the NEC was pipetted in each of 2 Processing Tubes (Processing Tubes are included in the Microsart® Bacteria Extraction kit) and labeled with "NEC 1" and "NEC 2".
2. 950 µl of the Matrix were pipetted in each of the tubes 1 to 8 (each of the tubes already contained 50 µl of unknown spiked sample) and mixed with a Vortex mixer.
3. The DNA of all 10 samples (2x NEC, 8x Unknown) were extracted following the protocol of the Microsart® Bacteria Extraction kit [2].
4. The PCR was setup following the protocol of the Microsart® ATMP Bacteria detection kit [3].
5. DNA extracts were analyzed in duplicates (Overview of sample number for DNA extraction and PCR setup is listed in Table 3).
6. For data analysis instructions in the manual were used.

Table 3:

Overview of sample number for DNA extraction and PCR setup

	DNA Extraction Microsart® Bacteria Extraction	PCR Analysis Microsart® ATMP Bacteria
NEC	2	4
Unknown	8	16
PCR Negative Control (NTC)	n.a.	2
PCR Positive Control (PC)	n.a.	2
	Σ 10	Σ 24

Results

The expected results are listed in Table 4. The acceptance criterion was fulfilled if all samples containing *Clostridium sporogenes* (ATCC 19404) at concentrations of LOD_{95} and 2x LOD_{95} have been detected as positive, at least 6 out of 8 NECs of each participant were negative, 2 out of 2 negative PCR controls have to be negative and 2 out of 2 PCR positive controls were positive for each participant.

Table 4:

Expected results of each beta test participant

Sample Code and content	Expected result
NEC (Negative Extraction Control)	Negative
1 (1/2 LOD_{95} *C. sporogenes*)	Positive or negative
2 (2x LOD_{95} *C. sporogenes*)	Positive
3 (LOD_{95} *C. sporogenes*)	Positive
4 (No spike, Negative Extraction Control)	Negative
5 (LOD_{95} *C. sporogenes*)	Positive
6 (No spike, Negative Extraction Control)	Negative
7 (2x LOD_{95} *C. sporogenes*)	Positive
8 (1/2 LOD_{95} *C. sporogenes*)	Positive or negative
NTC (No Template Control)	Negative
PC (Positive Control)	Positive

LOD_{95} *C. sporogenes* = 99 CFU (*C. sporogenes* is worst case organism with the highest LOD_{95})

Table 3:

Detail of the participants results

Sample Name		Participant 1		Participant 2		Participant 3		Participant 4		Participant 5	
		FAM	ROX	FAM	ROX	FAM	ROX	FAM	ROX	FAM	ROX
NTC	PCR NTC 1	Negative	Correct	Negative	Correct	Negative	Correct	Negative	Correct	Negative	Correct
	PCR NTC 2	Positive	Correct	Negative	Correct	Negative	Correct	Negative	Correct	Negative	Correct
	PCR PC 1	Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct
	PCR PC 2	Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct
NEC	NEC 1	Negative	Correct	Negative	Correct	Negative	Correct	Negative	Correct	Negative	Correct
		Negative	Correct	Positive	Correct	Negative	Correct	Negative	Correct	Negative	Correct
	NEC2	Negative	Correct	Negative	Correct	Negative	Correct	Negative	Correct	Negative	Correct
		Negative	Correct	Negative	Correct	Negative	Correct	Negative	Correct	Negative	Correct
Unknown	1/2 LOD (Samples 1 and 8)	Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct
		Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct
		Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct
		Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct
	LOD (Samples 3 and 5)	Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct
		Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct
		Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct
		Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct
	2x LOD (Samples 2 and 7)	Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct
		Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct	Positive	Correct
		Positive	Correct	Positive	Correct	Negative	Correct	Positive	Correct	Positive	Correct
		Positive	Correct	Positive	Correct	Negative	Correct	Positive	Correct	Positive	Correct
	NEC (Samples 4 and 6)	Negative	Correct	Negative	Correct	Negative	Correct	Negative	Correct	Negative	Correct
		Negative	Correct	Negative	Correct	Negative	Correct	Negative	Correct	Negative	Correct
		Negative	Correct	Negative	Correct	Negative	Correct	Negative	Correct	Negative	Correct
		Negative	Correct	Negative	Correct	Negative	Correct	Negative	Correct	Negative	Correct

Note: In this study all of the 1x LOD_{95} samples have been detected correctly. 4 out of 5 participants detected all 2x LOD_{95} samples positive. At least 6 NECs have been negative per participant. 1 participant detected a positive signal in one PCR NTC. All positive control reactions were positive.

Discussion

5 participants, representatives of the biopharmaceutical industry and hospital associated labs reported results. 4 out of 5 participants analyzed all the unknown samples correctly. Regarding that one of the highest concentrated sample was not detected by one participant, while lower titer were, it can be considered that this was due to a handling error and not due to the sensitivity of the Microsart® ATMP Bacteria kit. Samples 4 and 6 were designed as unknown negative controls containing no DNA or cell spikes at all. All labs identified those samples correctly as negative.

At least 6 negative extraction controls have been negative per participant therefore, fulfilling the acceptance criterion. One participant detected a positive signal in one PCR No Template Control. We conclude that this is most likely due to a pipetting error as all other samples have been detected as expected by this participant.

Emphasizing that the participants have been untrained users, guided by Sartorius user instructions only, employing various different cycler instruments and mostly with no dedicated DNA-free lab conditions, this results demonstrate the lab-to-lab precision and robustness of the assay.

In summary, the Microsart® ATMP Bacteria real-time PCR kit with prior DNA extraction using specifically designed Microsart® Bacteria Extraction has been shown to be a rapid and robust tool for the detection of total bacterial contamination. Especially in time sensitive applications such as the quality control of short shelf life products like ATMPs, CAR-T cells and other cellular therapeutics it contributes to a risk reduction and thus to patient safety as it allows for QC test results prior patient treatment.

References

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2. Instructions for use: Microsart® Bacteria Extraction, Sartorius, Prod. No. SMB95-2001, Ver.06 | 2018
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