Next Generation Cellular Therapeutic Technologies: Rapid Detection of Bacterial Contamination

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Sterility is a critical quality attribute (CQA) of cellular therapeutics. Since microbial contamination of cell therapy products can potentially result in the deaths of the 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 contamination can be ruled out with certainty, which is too long for short shelf life cellular therapeutics and 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 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 set up to meet requirements of the European Pharmacopeia chapter 5.1.6.

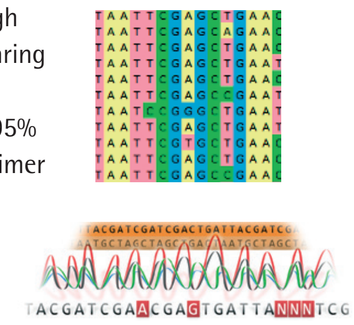
Beta Test | Lab-to-Lab Precision
In total 8 unknown samples were processed by 5 different participants belonging to the ATMP sector, federal institutes and hospitals.

Device Compatibility

The qPCR assay was tested on 4 different qPCR cyclers. Additionally other qPCR cyclers were tested during beta tests at customers facilities.

Bacteria Detection Range
Specific primer and TaqMan™ probes ensure the amplification of the highly conserved 16S rRNA coding region in the bacterial genome. Bacteria Detection Range was exemplary tested with 20 different species.

Sequence Alignment
Specific primer were selected through extensive in-silico analysis by comparing the primer sequences with genomic target sequences of bacteria. For > 95% of all known bacterial species the primer match the target sequence or have a maximum of three missmatches.



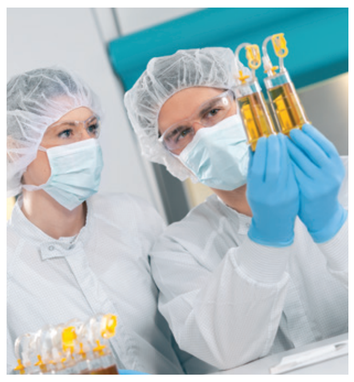
Validation

Robustness | Specificity | Sensitivity

Avoid Detection of Free DNA
The DNA extraction protocol discriminates free DNA from dead cells from the first step on. Due to a centrifugation step free DNA remains in the supernatant. Only the pellet containing intact cells is processed.

Cell Culture Spiked with Bacteria
At least 10 different mammalian cell culture samples in a relevant cell density of 10^6 cells/ml were spiked with bacteria to demonstrate highest sensitivity in various matrices.

Comparison to Compendial Sterility Test
In total 6 species were used for this comparison study. All species were tested by direct inoculation either in house or by an external contract lab at relevant concentrations of $2 \times LOD_{95}$, LOD_{95} and $LOD_{95}/2$ [cfu/ml].



Sample Matrix Effects | Cross Reactivity
Cross reactivity can be ruled out due to sequence alignment studies. In addition, at least 10 different mammalian cell culture samples at a relevant cell density were tested for cross reactivity and sample matrix effects.

LOD_{95} determination
Sensitivity testing is performed with 20 different bacterial species which have been selected in consultation with the German Federal Institute for Vaccines and Biomedicines. All tests were performed 6 or 2 times to have at least 24 or 8 results for each CFU concentration.

Spike DMEM + 5% FBS with bacteria					
99 CFU/ml	50 CFU/ml	10 CFU/ml	5 CFU/ml	2.5 CFU/ml	0 CFU/ml
▶4 aliquotes	▶4 aliquotes	▶4 aliquotes	▶4 aliquotes	▶4 aliquotes	▶4 aliquotes
DNA is extracted via Microsart® Bacteria Extraction. PCR is performed with 2 PCR positive controls and 2 PCR negative controls.					

Table 1: Test setup for LOD_{95} determination

CFU/ml		ct values (FAM)				Mean	Hit Rate
99	Run 1	32.51	32.11	31.73	32.27	32.15	8/8
	Run 2	32.16	32.40	32.17	32.03	32.19	
50	Run 1	32.93	34.20	33.76	34.00	33.72	8/8
	Run 2	34.62	33.36	33.36	33.44	33.69	
10	Run 1	36.11	35.97	36.00	35.62	35.92	8/8
	Run 2	35.02	36.28	34.64	34.47	35.10	
5	Run 1	35.03	37.25	36.62	37.05	36.49	8/8
	Run 2	36.17	37.39	36.30	36.27	36.53	
2.5	Run 1	36.78	No Ct	37.05	No Ct	36.91	6/8
	Run 2	36.73	36.88	35.54	36.85	37.25	

Table 2: Results for LOD_{95} determination of *Bacillus cereus*. The LOD_{95} for *Bacillus cereus* is 5 cfu/ml.

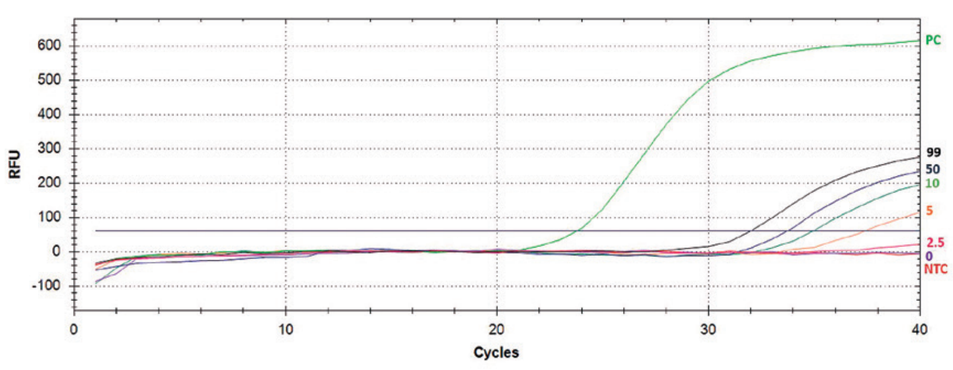


Figure 1: Amplification plot of different concentrations [CFU/ml] of *Bacillus cereus*

Summary and Outlook

The depicted scheme and results give an impression of the complexity of product validation required for a qPCR based bacterial detection kit for cellular therapeutics.
In addition to the classic sterility testing, a rapid qPCR based detection of bacterial contamination contribute to a risk reduction and therefore contribute to patient safety.

A respective assay to detect fungal contamination using the same technology and temperature profile as the bacteria assay will complete the portfolio, enabling simultaneous results of total bacteria and fungi within the same PCR run, within 3 hours instead of weeks. A rapid detection of such contaminants in cellular therapeutics with short shelf lives and especially autologous cell therapies is urgently needed prior administration to terminally ill patients.