Validation and Comparability of the BD BACTEC FX40 Device for Sterility Testing of CAR-T Drug Products: A Comprehensive Study

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Introduction

Sterility testing plays a crucial role in the release testing of CAR-T therapeutic products, considering their personalized nature and the challenge of preserving them over extended periods. Novel systems are currently under development to expedite the detection of contamination. Among these, the BACTEC FX40 system underwent evaluation in comparison to the conventional compendial sterility test method of direct inoculation.

From a regulatory standpoint, the validation of rapid sterility methods requires demonstrating both sufficient sensitivity and the suitability of the sample matrix under the stringent conditions mandated by the Pharmacopoeia. In general, the implementation of rapid sterility tests should be justified through a risk-based approach and in consultation with regulatory authorities.

Methods

Nine microbial reference strains (aerobic and anaerobic bacteria, and fungi) were inoculated into the BACTEC vials (Bottle Plastic Bactec Std Aerobic/F 50Pk or Bottle Plastic Bactec Lytic/10 Anaer/F) in various concentrations (100, 50, and 10 CFU/vial), together with FOS supplement. Concentration 100 CFU was tested on three batches of frozen CAR-T product. Concentrations of 50 CFU and 10 CFU were tested in triplicates of each microorganism on one batch of frozen CAR-T drug product. Positive controls of microorganisms and negative control of selected media, FOS supplement, and CAR-T drug products were prepared.

Testing by the compendial method (Ph. Eu. 2.6.1) was carried out in parallel.

All BACTEC vials were cultivated in the BACTEC FX40 at temperature 35 ± 1,5°C up to 14 days, or until detection of microbial growth (positive status in the device). All samples for compendial sterility testing were cultivated at temperature 30 - 35 °C and 20 - 25 °C up to 14 days, or until detection of microbial growth.

Three in-house isolates (aerobic bacteria) were also tested in concentration 100 CFU/vial without CAR-T product.

Figure 1: Module of BACTEC FX40.

Results

We optimized the culture conditions, including the addition of FOS supplement, and the technique of vial inoculation into the BACTEC vials, particularly for anaerobic strains. The growth in positive controls and in tested samples with microorganism was detected for all tested strains and was comparable. All types of negative control were without growth after 14 days.

Comparability with the compendial method (inoculation of 100 CFU): All tested microbial strains (9 reference strains and 3 in-house isolates from the clean room environment) showed positive results within four days in BACTEC FX40, in a comparable time frame to the compendial method.

Sensitivity experiment: Samples were inoculated with 10 or 50 CFU, in triplicates for all conditions. BACTEC FX40 confirmed sensitivity as low as 10 CFU for all tested microoganisms, which was even superior to the compendial methods where no growth was observed in the case of *Pseudomonas aeruginosa*.

Tested sample	Microorganism 100 CFU	BACTEC FX40	Direct inoculation
CAR-T-1	*Clostridium sporogenes*	18 h 15 min	1 day
CAR-T-2		15 h 13min	1 day
CAR-T-3		15 h 3 min	1 day
CAR-T-1	*Cutibacterium acnes*	3 days 17 h 37 min	5 days *
CAR-T-2		3 days 3 h 8 min	2 days
CAR-T-3		2 days 20 h 37 min	5 days *
CAR-T-1	*Staphylococcus aureus*	14 h 34 min	3 days *
CAR-T-2		14 h 5 min	3 days *
CAR-T-3		14 h 8 min	3 days *
CAR-T-1	*Streptococcus pyogenes*	13 h 35 min	3 days *
CAR-T-2		13 h 16 min	3 days *
CAR-T-3		13 h 40 min	3 days *
CAR-T-1	*Brachybacterium nesterenkovii*	1 day 8 h 38 min	3 days *
CAR-T-2		1 day 8 h 39 min	3 days *
CAR-T-3		2 days 23 h 40 min	3 days *
CAR-T-1	*Pseudomonas aeruginosa*	15 h 6 min	3 days *
CAR-T-2		15 h 3 min	3 days *
CAR-T-3		15 h 4 min	3 days *
CAR-T-1	*Bacillus subtilis*	10 h 49 min	2 days
CAR-T-2		12 h 7 min	1 day
CAR-T-3		11 h 48 min	1 day
CAR-T-1	*Candida albicans*	1 day 2 h 8 min	2 days
CAR-T-2		1 day 3 h 46 min	1 day
CAR-T-3		23 h 35 min	1 day
CAR-T-1	*Aspergillus brasiliensis*	1 day 11 h 5 min	3 days
CAR-T-2		2 days 10 h 50 min	2 days
CAR-T-3		1 day 20 h 21 min	2 days

Table 1: Comparability of the BACTEC rapid sterility method and compendial method in the suitability test. Time to detection is recorded for BACTEC FX40.

Tested sample	Microorganism 10 CFU	BACTEC FX40	Direct inoculation
CAR-T -1 I	*Clostridium sporogenes*	16 h 8 min	1 day
CAR-T -1 II		17 h 36 min	1 day
CAR-T -1 III		16 h 4 min	1 day
CAR-T -1 I	*Cutibacterium acnes*	3 days 20 h 11 min	6 days *
CAR-T -1 II		3 days 12 h 6 min	6 days *
CAR-T -1 III		3 days 23 h 8 min	6 days *
CAR-T -1 I	*Staphylococcus aureus*	16 h 4 min	2 days
CAR-T -1 II		16 h 5 min	2 days
CAR-T -1 III		15 h 38 min	2 days
CAR-T -1 I	*Streptococcus pyogenes*	10 h 19 min	3 days *
CAR-T -1 II		10 h 25 min	3 days *
CAR-T -1 III		10 h 39 min	3 days *
CAR-T -1 I	*Brachybacterium nesterenkovii*	1 day 11 h 34 min	3 days *
CAR-T -1 II		1 day 10 h 4 min	3 days *
CAR-T -1 III		1 day 11 h 38 min	3 days *
CAR-T -1 I	*Pseudomonas aeruginosa*	15 h 24 min	no growth
CAR-T -1 II		17 h 34 min	no growth
CAR-T -1 III		18 h 36 min	no growth
CAR-T -1 I	*Bacillus subtilis*	13 h 3 min	2 days
CAR-T -1 II		12 h 53 min	2 days
CAR-T -1 III		12 h 36 min	2 days
CAR-T -1 I	*Candida albicans*	1 day 1 h 8 min	2 days
CAR-T -1 II		1 day 9 h 36 min	2 days
CAR-T -1 III		1 day 7 h 31 min	2 days
CAR-T -1 I	*Aspergillus brasiliensis*	3 days 5 h 43 min	3 days
CAR-T -1 II		3 days 11 h 27 min	3 days
CAR-T -1 III		3 days 20 h 6 min	3 days

* The compendial samples were evaluated only on working days. The positivity could have been detected from one/two days earlier.

Table 2: Assessment of the method sensitivity. 10 CFU inoculation in triplicate in one batch of CAR-T product.

Micro 100 CFU	BACTEC FX40	Direct inoculation
Kocuria rhizophila	1 day 4 h	1 day
Moraxella osloensis	2 days 12 h	3 days
Bacillus cereus	8 h	1 day

Table 3: Comparability of the BACTEC rapid sterility method and compendial method in three in-house isolates.

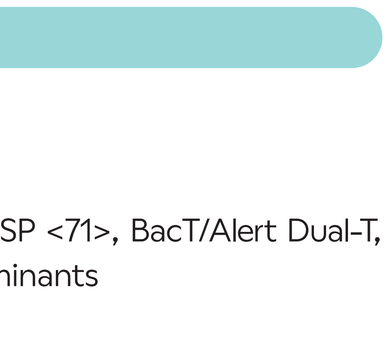

Figure 2: BACTEC vial with *Aspergillus brasiliensis*.

Discussion

The optimized rapid sterility test using the BD BACTEC FX40 device demonstrated high sensitivity in detecting low levels of bacterial and fungal contamination in CAR-T drug products. Moreover, all microorganisms detected by the BACTEC FX40 were successfully grown in subsequent subcultures, validating the reliability of the method. Nevertheless, we encourage the testing of other cell therapy-specific environmental bacterial or fungal species. Strains from the environment that necessitate lower culture temperatures can cause challenges for cultivation. One effective strategy to overcome this hurdle, as recommended by England *et al.*, involves conducting parallel testing using SDA plates. To ensure maximum safety, a conservative approach of combining BACTEC testing with the compendial method is recommended for conditional product release after five days of BACTEC testing, followed by the compendial sterility test (after 14 days) for the final release.

Conclusion

The rapid sterility test using the BD BACTEC FX40 device proved to be a robust and reliable method for rapid sterility testing of frozen CAR-T drug products. The method sensitivity was assessed to be 10 CFU and all tested microorganisms were detected within four days.

The method fullfilled all the criteria for comparability to the compendial method as requested by Ph. Eu. and USP. Its rapid detection and comparability to the compendial sterility test make it an attractive option for routine testing.

References

European Pharmacopoeia Ph. Eu., Chapters 2.6.1, 2.6.27

United States Pharmacopoeia USP, Chapters <71>, <1071>

England *et al.* 2019: Comprehensive Evaluation of Compendial USP <71>, BacT/Alert Dual-T, and Bactec FX for Detection of Product Sterility Testing Contaminants