REPLACING TRADITIONAL ASEPTIC PROCESS SIMULATIONS WITH QUALIFICATION OF CELL AND GENE THERAPY SUPERNATANT

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ABSTRACT

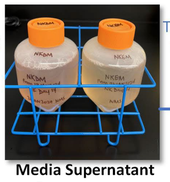
TSB (Tryptic Soy Broth) is the standard growth media used in Aseptic Process Simulation (APS) for Cell and Gene Therapy (CGT). Cell Therapy drug products are aseptically manufactured, but the final product consists of viable cells that cannot be sterilized, and these cells require longer incubation times in nutrient rich media. The unique challenges of cell therapy manufacturing highlight the need for new and innovative approaches for aseptic process simulations. This study was designed to determine if the cell culture media used for manufacturing provides the necessary nutrients to promote the growth of USP Growth Promotion (GP) organisms similarly to TSB. This hypothesis was tested on the "supernatant" also known as "spent media" collected from the manufacturing process. It challenges two types of cell culturing medium (Media 1 and Media 2) per the FDA requirements for APS conditions (Test A) versus process-use conditions (Test B) against the USP-GP organisms. Through the execution of this study, a self-validating process was demonstrated to allow for new criteria in CGT to be established that fulfill the APS re-qualification requirement with each manufacturing run. This reduction of APS re-qualifications increases the efficiency of operations, reduces manufacturing downtime and can make drug product more readily available to patients.

OBJECTIVES

The goal of this study is to demonstrate that a manufacturing process that is contamination-indicating could negate the need for an APS every 6 months, since the manufacturing process can serve as a re-qualifying APS run each time it is executed. Tests A-B were challenged to assess that the supernatant from cell culture media (Media 1 & Media 2) was capable of supporting growth of the USP-GP organisms after being exposed to the same incubation parameters required for the FDA guidance APS conditions (Test A) and process use conditions (Test B).

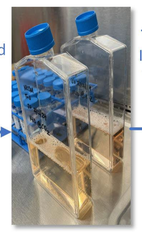
METHODS

Media Supernatant Exposure to Test A and Test B Conditions



Media Supernatant
Media 1: Supernatant from day 14
Media 2: Supernatant from day 21

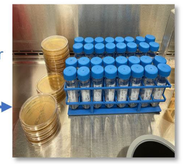
Transferred Media to Culture Flasks



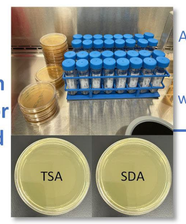
Test A (APS Conditions) Incubated at 30-35°C for 7 days then transferred to 20-25°C for 7 days

Test B (Process Use Conditions) Incubated at 37C°/+5% CO_2/+5% 0_2 for 14 days

Aliquoted 10mL of Media in Into 50mL Tubes in Duplicate for Each Test Organism

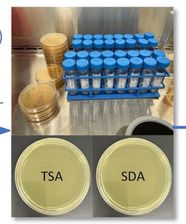


Growth Promotion Method for Test A and Test B Media



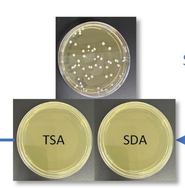
Spiked The Test Media 10mL Aliquots and Tryptic Soy Agar (TSA) and Sabouraud Dextrose Agar (SDA) Control Plates in Duplicate with USP Organisms at <100cfu/mL

Lyophilized Cultures Used:
S.aureus, B.spizizenii, E.coli, P.aeruginosa, A.brasiliensis, C.albicans and *S.epidermidis (Environmental Isolate)*



Controls: Incubated Fungi at 20-25°C for <5 Days and Bacteria at 30-35°C for <3 days
Test Media: Incubated Bacteria and Fungi at 30-35°C for <3 Days

Enumerated the Control Plates and Subcultured Fungi to SDA and Bacteria to TSA



Incubated Fungi at 20-25°C for <5 Days and Bacteria at 30-35°C for <3 days

Gram Stained Test Samples and Performed Microscopic Evaluation

RESULTS

Test A and Test B (Media 1)

Culture	Replica #1 Test A (Growth/No Growth)	Replica #2 Test A (Growth/No Growth)	Replica #1 Test B (Growth/No Growth)	Replica #2 Test B (Growth/No Growth)
Sa+	Growth	Growth	Growth	Growth
Bs+	Growth	Growth	Growth	Growth
Ec+	Growth	Growth	Growth	Growth
Pa+	Growth	Growth	Growth	Growth
Se+	Growth	Growth	Growth	Growth
Ab+	Growth	Growth	Growth	Growth
Ca+	Growth	Growth	Growth	Growth
N/C	No Growth	No Growth	No Growth	No Growth

Test A and Test B (Media 2)

Culture	Replica #1 Test A (Growth/No Growth)	Replica #2 Test A (Growth/No Growth)	Replica #1 Test B (Growth/No Growth)	Replica #2 Test B (Growth/No Growth)
Sa+	Growth	Growth	Growth	Growth
Bs+	Growth	Growth	Growth	Growth
Ec+	Growth	Growth	Growth	Growth
Pa+	Growth	Growth	Growth	Growth
Se+	Growth	Growth	Growth	Growth
Ab+	Growth	Growth	Growth	Growth
Ca+	Growth	Growth	Growth	Growth
N/C	No Growth	No Growth	No Growth	No Growth

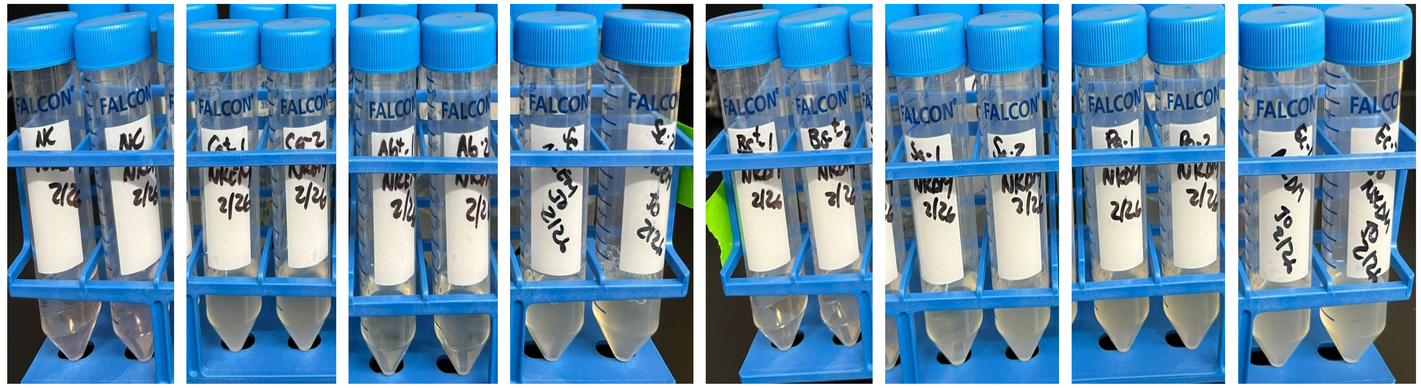
Control Plates

Culture	Control (Replica 1)	Control (Replica 2)	Control Average (≤100cfu/plate)
Sa+	27	31	29
Bs+	27	25	26
Ec+	6	4	5
Pa+	13	11	12
Se+	27	28	28
Ab+	31	43	37
Ca+	20	22	21
N/C	0	0	0

Legend

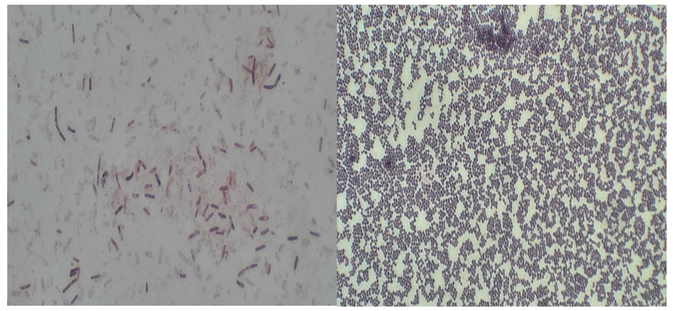
Abbreviation	Organism
Sa+	*S. aureus*
Bs+	*B.spizizenii*
Ec+	*E.coli*
Pa+	*P.aeruginosa*
Se+	*S.epidermidis*
Ab+	*A.brasiliensis*
Ca+	*C.albicans*
N/C	Negative Control

USP Organism Growth in Test Media

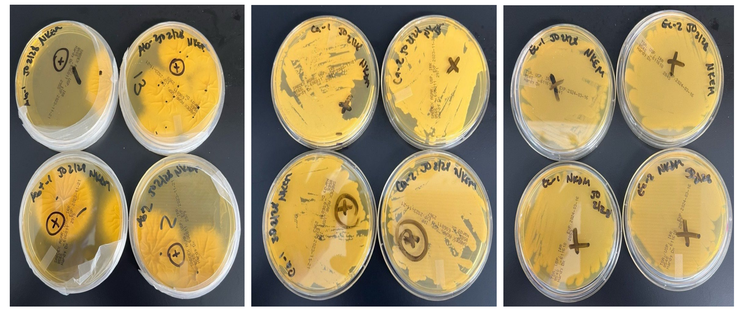


Negative Control | *C.albicans* | *A. brasiliensis* | *S. epidermidis* | *B. spizizenii* | *S. aureus* | *P. aeruginosa* | *E. coli*

Gram Stain of Sub-cultures from Spiked Test Media



Sub-cultures from Spiked Test Media



CONCLUSION

Tests A-B demonstrated that the supernatant from cell culture media (Media 1 and Media 2) is capable of supporting growth of USP GP organisms after being exposed to the same incubation parameters per the FDA requirements for APS conditions (Test A) and process use conditions (Test B). Based on these findings, it can be concluded that the cell culture media (Media 1 and Media 2) for this cell therapy process can recover growth during the manufacturing process, if contamination were ever present. These data suggest that each manufacturing run behaves as an APS re-qualification run. By demonstrating a self-validating process, new criteria for APS in CGT can be established that allows the APS re-qualification requirement to be fulfilled with each manufacturing run.

REFERENCES

- Eudralex Annex 4 – Guidelines on GMP's specific to ATMPs
- FDA Guidance: Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Process (2004)
- USP <62> Microbial Examination of non-sterile products: Microbial Enumeration Test
- USP<61> Examination of non-sterile products: Tests for Specified Microorganisms