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S/C.NEST™ | Validation of the aseptic culture environment in S.NEST and C.NEST incubation chambers by an OECD GLP- accredited institution

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Abstract

Maintaining an uncontaminated environment is crucial for the successful cultivation of cells or microorganisms. The S.NEST and C.NEST microbioreactors, with their patented microfluidic technology, provide reciprocating mixing in each well of 24- or 96-well plates by harnessing airflow from both CO_2 cylinders and the surrounding atmosphere. Despite this air being filtered through a HEPA filter, akin to standard incubators, to preemptively address any potential concerns of contamination via airflow, we commissioned Weshin Inspection Tech Co., Ltd., an OECD GLP accredited institution (GLP0069), to conduct a comprehensive validation of the sterility of the S.NEST and C.NEST systems. The result of rigorous total bacteria testing performed by Weshin Inspection Tech affirmed that the environment within S.NEST and C.NEST remains uncontaminated when operated in accordance with aseptic technique guidelines. This effectively demonstrates that, with proper adherence to aseptic procedures, the S.NEST and C.NEST microbioreactors are reliable and free of

concerns regarding atmospheric and instrumental contamination.

Introduction

The C.NEST and S.NEST microbioreactors from CYTENA BPS represent a leap forward in cell and microorganism cultivation technology, offering a meticulously controlled environment crucial for sensitive cultivation processes. Each microbioreactor is equipped with four independent incubation chambers that precisely regulate critical growth parameters, such as temperature, CO_2 levels, and humidity. These advanced units stand out for their patented reciprocating mixing mechanism, which enables uniform mixing across each well of 24 or 96-well plates, offering shake-flask-like conditions at microscale for the early stages of cell line development (CLD), process development (PD) and more. This feature is particularly beneficial in shortening cell doubling time, boosting cellular viability, and easing the transition to suspension cultures for a diverse range of cells, including mammalian cells like CHO and various microorganisms.

The innovative design of these microbioreactors incorporates a unique mixing mechanism utilizing air pressure sourced from both CO_2 cylinders and an environmental air inlet port. Before entering the instrument, both air and CO_2 undergo stringent filtration through HEPA filters, ensuring their purity. In addition, each chamber is enhanced with a UV light sterilization function, adding an extra layer of defense against potential contamination. Recognizing the critical need for sterility in cell culture, CYTENA BPS has actively pursued validation and reinforcement of the sterile environment provided by the S.NEST and C.NEST microbioreactors.

As a proactive measure to address and alleviate any concerns regarding contamination through air input, CYTENA BPS commissioned Weshin Inspection Tech Co., Ltd., an OECD GLP accredited institution (GLP0069), to perform an experiment. The goal was to validate the effectiveness of the integrated HEPA filters and UV sterilization, as well as to evaluate the overall design and operational reliability of the microbioreactors in maintaining sterility. The outcome of the total bacteria testing indicated that, when proper aseptic procedures are followed, the S.NEST and C.NEST microbioreactors are contamination-free, validating the robustness and effectiveness of their design and sterilization processes.

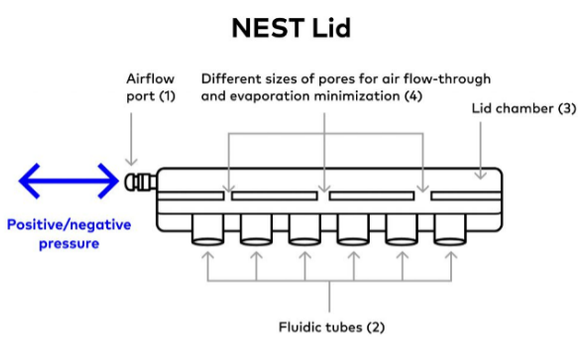
Material and Methods

A. Preparation of C.NEST and S.NEST:

Before beginning the experiment, new HEPA filters were installed in both C.NEST and S.NEST. The installation was followed by thorough sterilization of all accessible interior surfaces of the designated incubation chambers using 75% ethanol. Subsequently, the water reservoirs were filled with sterilized double-distilled water (ddH_2O). To ensure optimal sterility, each unit underwent a 15-minutes UV sterilization cycle, sterilizing the culture chambers and the ddH_2O.

B. Sample Preparation in Biosafety Cabinet (BSC):

Within the controlled confines of a biosafety cabinet, two 24-well plates were prepared in adherence to the aseptic technique guidelines for C.NEST and S.NEST systems. Each well was carefully filled with 1,600 µL of antibiotic-free Gibco™ CD Hybridoma Chemically Defined Medium (Thermo Fisher Scientific). The plates were then covered with lids designed to permit airflow into each well, facilitating reciprocating mixing.



C. Cultivation Conditions:

We placed one 24-well plate into one designated incubation chamber for C.NEST and one for S.NEST. Following the aseptic technique guidelines, we wiped the surface of the NEST lid/plate set with 75% ethanol before placing it into the chamber. Both plates were then cultivated under identical conditions: a constant temperature of 37°C, a CO_2

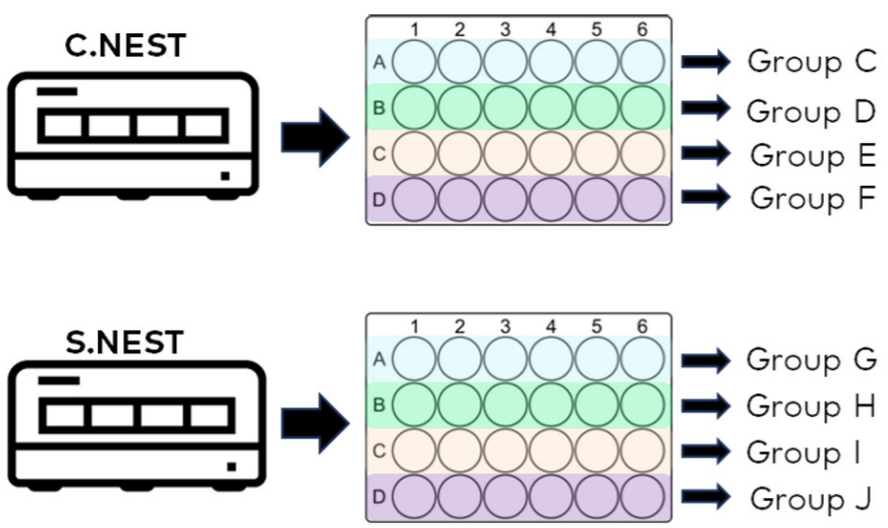
level of 5.0%, and continuous mixing at a rate of 50 seconds per cycle over a 14-days period.

D. Sample Collection for Microbial Testing:

Upon completion of the cultivation period, samples were collected from the medium in each well according to the specified grouping (illustrated in the diagram below). These samples were carefully packaged and sent to Weshin Inspection Tech Co., Ltd. for comprehensive total bacterial testing. This critical step was undertaken to confirm the aseptic conditions and overall sterility of the C.NEST and S.NEST systems.

E. Total Bacterial Test:

The total bacterial tests were conducted by Weshin Inspection Tech Co., Ltd. Ten groups were included in the testing: one positive control group (Group A) where bacteria were deliberately inoculated in the Weshin laboratory to confirm the assay's effectiveness, and one negative control group (Group B) using sterile ultrapure water prepared by the Weshin laboratory to check for any procedural contamination. Groups C to F comprised samples from C.NEST, while Groups G to I included samples from S.NEST. The aerobic plate count (APC) for all test groups was measured in CFU/mL to determine the bacterial load in the assay.



Result and disccusion

This study validated the sterility of the S.NEST and C.NEST microbioreactors with CD Hybridoma Chemically Defined Medium in 24-well plates. Following a 14-day cultivation period, samples were collected and subjected to total bacterial testing according to standard microbial testing procedures. The groups included a positive control (Group A) to verify the experiment's effectiveness, a negative control (Group B) to assess procedural contamination, and various groups from both C.NEST (Group C-F) and S.NEST(Group G-J) microbioreactors representing different sections of the 24-well plate.

The reported aerobic plate count across all test groups, excluding the positive control used for validation purposes, was 0 CFU/mL, indicating no detectable bacterial growth and thus confirming the sterility of the cultures.

Aerobic plate count					
Group	**A** Positive Control	**B** Negative Control	**C** C.NEST(A1-A6)	**D** C.NEST(B1-B6)	**E** C.NEST(C1-C6)
Aerobic plate count (CFU/mL)	>100	0	0	0	0
Group	**F** C.NEST (D1-D6)	**G** S.NEST(A1-A6)	**H** S.NEST(B1-B6)	**I** S.NEST(C1-C6)	**J** S.NEST(D1-D6)
Aerobic plate count (CFU/mL)	0	0	0	0	0

Table 1. Aerobic plate count of positive control (A), negative control (B), and samples from C.NEST (C-F) and S.NEST(G-J).

Conclusion

This validation study has shown that the C.NEST and S.NEST microbioreactors sustain fully sterile environments, an essential requirement for cell and microorganism cultivation. An external OECD GLP accredited institution, Weshin Inspection Tech Co., Ltd., was able to verify that all test samples from this study produced an aerobic plate count of 0 CFU/mL. To conclude, the design of C.NEST and S.NEST assures sterility, when recommended aseptic technique guidelines are adhered to.

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