Application Note

CELLENION
A BICO COMPANY

cellenONE® Sterile Condition Operation

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Abstract

Single cell methods require extreme cleanliness to avoid exogenous contamination, as a minute microbial contamination can ruin a cell cloning procedure and jeopardize a single cell sequencing approach. We evaluated axenic (germ free) conditions with cellenONE® comparing two systems: a regular cellenONE® X1 and a cellenONE® X1 BSC (version of the instrument mounted in a BSC environment).

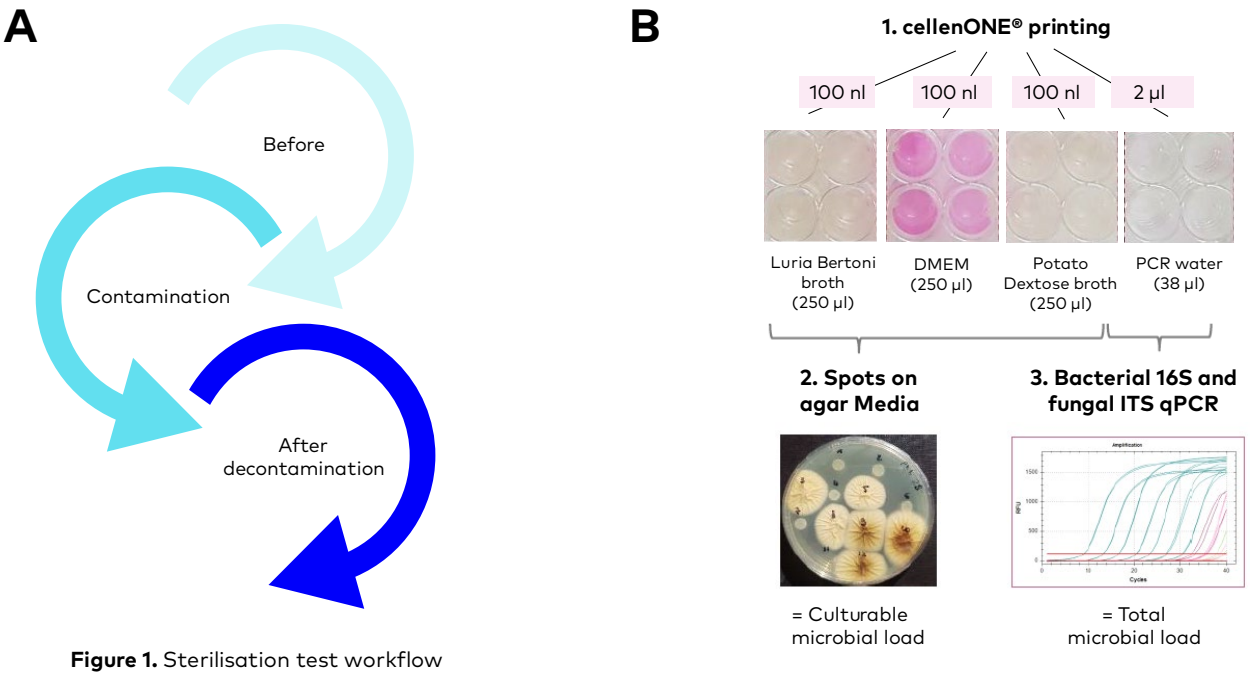


Figure 1. Sterilisation test workflow

Materials and methods

Sterilization experiments consisted of 3 conditions (Fig. 1A), where the dispense was performed for each phase (Fig. 1B); 1) Before contamination: in **routinely clean** (H_20, EtOH, detergent) cellenONE® systems. 2) Contamination: in **intentionally contaminated** systems. Environment generated by aspirating and dispensing a concentrated suspension of viable *E. coli* bacteria and *A. niger* fungal spores with the cellenONE® nozzle. 3) After decontamination: in **sterilized** systems.

1. Drops were **cellenONE® dispensed in sterile growth media** (LB, DMEM and PDB for bacterial, human and fungal cells, respectively, 100 nL in 250 µL) and **PCR water** (2 µL in 38 µL).
2. Inoculated media were incubated (48h, 37°C) then spotted on **agar plates**, to assess for **culturable** bacterial and fungal load.
3. Inoculated PCR water was used as template for **real-time PCR** (qPCR) targeting universal bacterial 16S ribosomal genes and fungal ITS genes, to assess **total** microbial load.

Results and discussion

In **routinely clean** cellenONE® X1, 1 out of 48 wells had a bacterial contamination, none in X1 BSC.

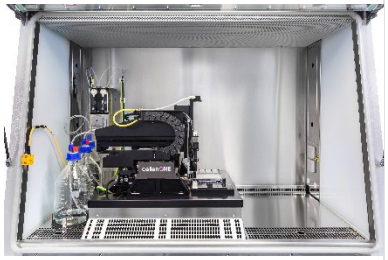
All **positive control** wells (intentionally heavy microbial contamination) were contaminated as expected and flushing the nozzle with water was not sufficient to remove the contamination.

cellenONE® X1 and cellenONE® X1 BSC **became germ-free** again after sciCLEAN8 and Sterilization tasks, respectively.

In the X1, the sterilization task* induces temporary sterile conditions. The X1 BSC is an operational germ-free environment such that detergent-based washing, sciCLEAN8, is sufficient to prevent contamination.

B: bacteria, **F**: fungi,
Cult. and **Tot**.: culturable and total microbial load
+++ : positive control
++ : major contamination (>1/12 wells)
+ : minor contamination (1/12 wells)
- : no contamination

X1



X1 BSC

			Cult.	Tot.	Cult.	Tot.
Before		B	+	-	-	-
		F	-	-	-	-
Contamination	Microbial suspension	B	+++	+++	+++	+++
		F	+++	+++	+++	+++
	After flushing nozzle	B	++	++	+	-
		F	++	-	-	+
After decontamination	sciCLEAN8	B	+	-	-	-
		F	+	-	-	-
	Sterilization Task*	B	-	-	-	-
		F	-	-	-	-

** A sequence of nozzle washing with 0.5 % sodium hypochlorite, 3% hydrogen peroxide and 70% ethanol*

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