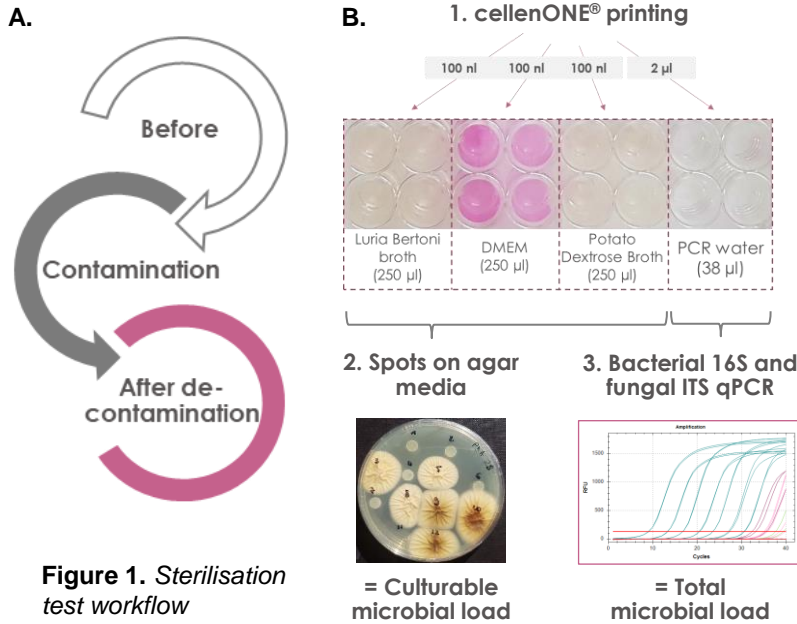


Application Note:

cellenONE® Sterile Condition Operation

Single-cell methods require extreme cleanliness to avoid exogenous contamination, as a minute microbial contamination can ruin a cell cloning procedure and jeopardise a single-cell sequencing approach. We evaluated axenic (germ-free) conditions with cellenONE®, comparing two systems: the integrated F1.4 unit and an X1 mounted in a BSC environment.



Materials and Methods

Sterilisation experiments consisted of 3 conditions (Fig. 1A), where the dispense was performed for each phase (Fig. 1B); 1) Before contamination: in **routinely clean** (H₂O, 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 **sterilised** 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.

Figure 1. Sterilisation test workflow

Results

In **routinely clean** F1.4 cellenONE®, 1 out of 48 wells had a bacterial contamination, none in X1 cellenONE® mounted in 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.

X1 mounted in BSC and F1.4 **became germ-free** again after sciCLEAN and Sterilisation tasks, respectively.



In the F1.4, the sterilisation task* induces temporary sterile conditions. The X1 mounted in a 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

		F1.4		X1 in BSC		
		Cult.	Tot.	Cult.	Tot.	
Before	B	+	-	-	-	
	F	-	-	-	-	
Contamination	Microbial suspension	B	+++	+++	+++	
		F	+++	+++	+++	
	After flushing nozzle	B	++	++	+	-
		F	++	-	-	+
After decontamination	sciCLEAN8	B	+	-	-	
		F	+	-	-	-
	Sterilisation task*	B	-	-	-	-
		F	-	-	-	-

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