Validation strategy

ASD in a CDMO BSL-3

devea

NAOBIOS, a GMP certified CDMO

Naobios is a **GMP-certified** French company specialising in **bioprocess development** projects, in particular viral products. This CDMO works with **live viruses** or **GMOs**, in aseptic conditions, in laboratory volumes of up to 200L in GMP production. The projects, and therefore the products, change regularly. Added to this is the nature of the viruses handled, which means that the **risk of biocontamination is particularly high**, and disinfection is subject to specific attention and monitoring.

In these **BSL-2** and **BSL-3** premises, **classified C to A**, Airborne Surface Disinfection (ASD) is carried out at the end of each campaign, approximately every two to three months, and for a maximum of every six months. The **configuration of the work area changes** with each project: equipment required, footprint and volume, etc. For each configuration, the need for a **new performance qualification** (PQ) is assessed on the basis of a **risk analysis** to ensure the **effectiveness of the end-of-campaign ASD** before working with a new biological material (virus). In all cases, the disinfection **PQ is revalidated every two years** at the latest, or sooner in the event of a major change.

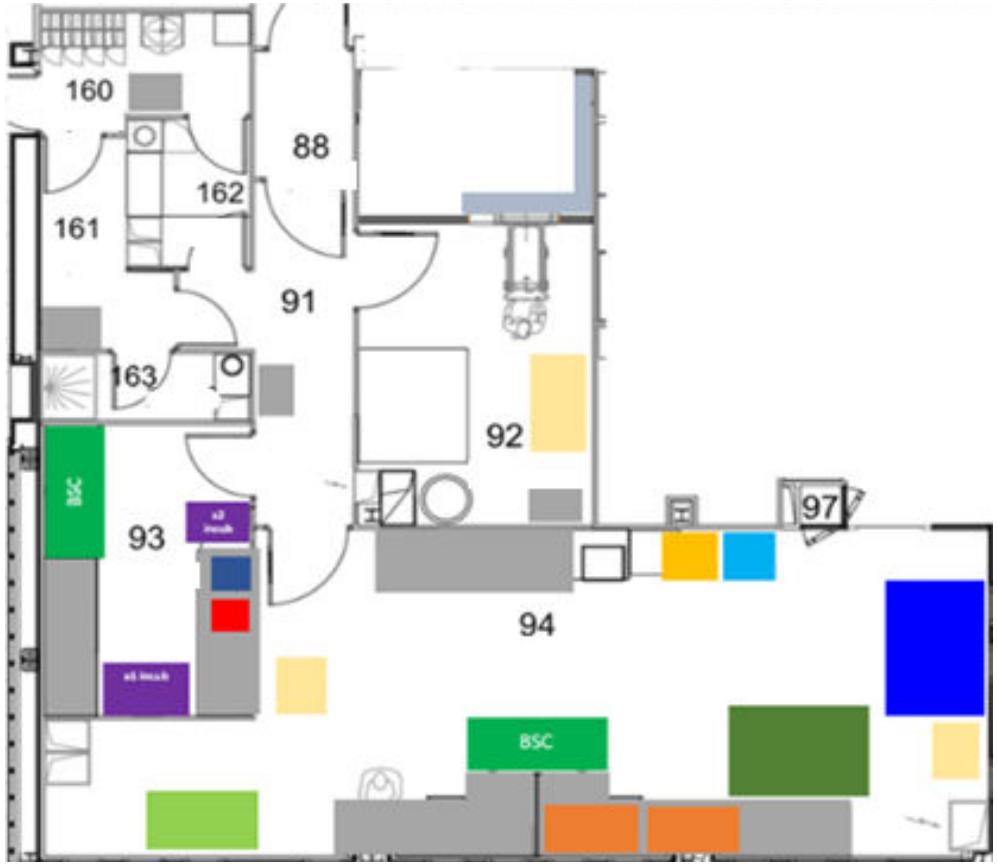
Project background

The project presented here is located in the "CM4" production area, a **BSL-3 zone measuring 270 m3** and comprising a **140 m3 production room**, a **31 m3 extraction room** with a microbiological safety cabinet (**BSC**) directly connected to the ventilation circuit (fume hood) and **changing rooms**, **airlocks** and additional rooms (see plan of the area, graph 1). The disinfection equipment, a VHP type, was ageing and obsolete, so a replacement was needed. At the same time, other ASD solutions were studied; the users' specifications included the following::

- ✔ disinfection efficacy at 6 log10 spore reduction level,
- ✔ staff safety,
- ✔ harmlessness to the equipment and structure of the Controlled Atmosphere Zone,
- ✔ easy to install,
- ✔ data traceability and compliance.

Project background

Since 2009, **Devea** has been offering an innovative, patented technology for **ASD by nebulisation** with low-concentration hydrogen peroxide (7.4%): this solution - Phileas® range of devices and O2SAFE 7.4® biocide - has received its **Marketing Authorisation** (MA) from 2019 in France. **Centrifugation** technology produces a fine droplet size (5 to 10µm) that is **uniform** throughout the diffusion process, and evenly distributed throughout the space. Treatment with this 7.4% biocide is non-corrosive and leaves no residues. It generates an **H2O2 concentration** in the space of **100 to 120ppm on average**, i.e. 5 to 10 times less concentrated than hot vapor technology with a 35% biocide. Phileas® devices are **'plug-and-play'** and extremely **easy** to set up and use, over a wide range of environmental temperature and humidity conditions. Lastly, the **MyPhileas application** solution enables the devices to be controlled remotely and to generate pdf **diffusion reports**, in compliance with **GMP Annex 11 and 21 CFR Part 11**.



Graph 1: configuration and equipment of the "CM4" zone. Description of room : 160: grey personnel airlock; 162: white personnel airlock; 161: exit personnel airlock; 163: safety shower; 88: equipment airlock; 91: corridor; 92: technical room; 93: production laboratory 1; 94: production laboratory 2; 97: pass-through.

Project background

	Phileas® Genius	Phileas® ONE	Phileas® 250
Image			
Volume disinfected	0.5-5 m3 (BSC, isolator)	10-165 m3 (room, laboratory, operating theatre)	20-800 m3 (corridor, multi-room, large areas)
Diffusion module	1 module	1 module	2 modules
Diffusion flowrate	400 mL/hr	1,2 L/hr	3 L/hr
Biocide capacity	250 mL	2 L	10 L
Comment	On battery	-	Integrated weight sensor

Table 1: Characteristics of the models in the Naobios fleet

Cycle development

Once the equipment had been qualified (installation and operational qualifications), the **cycle development** phase began for both parts of the zone. The choice was made to test night-time cycles, in line with the site's habits:

- start of disinfection at the **end of the day** (end of activity in adjacent areas),
- **long contact time**,
- restarting the ventilation (HVAC) at the end of the night, so that operations can resume on the following morning.

This **organisation** means that **operations are not interrupted**, and disinfection can be carried out entirely in down time.

Cycle development

Graph 2 shows the **room configuration** in zone CM4, as well as the **Phileas® devices placement** for the ASD in parts 1 and 2. For these cycle developments, the biological indicators (BI) are ***Geobacillus stearothermophilus*** 6log10 in a Tyvek envelope (Mesa Labs), tripled at each point, accompanied by a **chemical indicator** (CI) at each point. The indicators in the biosafety cabinet in room 93, subject to additional validation, are **triscale** (log10 4, 5 and 6 indicators in the same Tyvek envelope, Mesa Labs), which makes it possible to check the **disinfection log achieved**. The number and positioning of the indicators in the rooms had previously been the subject of a **risk analysis**, and there was no need to modify them for this project (see Table 2).

CM4 Part 1 CM4 Part 2

Graph 2: Setting up the ASD for parts 1 and 2 of the "CM4" zone: equipment and indicators

Cycle development

IB/IC position Part 1	IB/IC position Part 2	IB/IC position BSC room 93
1: in the centre, on the bench	1: on the safety shower tray	1: exhaust filter outlet
2: at the centre of the BSC	2: on the equipment	2: left wall of the plenum
3: under the Phileas® 250	3: on the ground in front of the equipment	3: right wall of the plenum
4: at the centre of the equipment	4: under the Phileas® 250	4: left-hand side of worktop
5: on the return grid (part 93)	5: on the ground, staff exit airlock	5: right-hand wall of worktop
6: at the centre of the equipment	6: on the ground, grey personnel airlock	6: under the worktop on the left
7: in the equipment	7: on the floor, white personnel airlock	7: under the worktop on the right
8: in the equipment	8: on the ground, equipment lock	
9: on the equipment		
10: at the centre of the equipment		
11: at the centre of the equipment		
12: behind the return grid (part 94)		
13: no IB 13 – numbering mistake		
14: at the centre of the equipment		
15: at the centre of the equipment		

Table 2: Positioning of chemical and biological indicators in Parts 1 and 2 and in the BSC (Room 93)

Cycle development

The strategy for disinfecting the BSC in room 93 was discussed at length (the only BSC directly hooked on to the HVAC on site - the other BSCs are not connected to the HVAC): the BSC, even when shut down, is ventilated by the HVAC. In order to disinfect it, the HVAC has to be switched off, which means shutting down the entire area. **Disinfecting the BSC therefore means disinfecting the entire zone**.

The ASD sequence for change-over campaigns is as follows:

- **cleaning** the area and equipment
- 4pm: **installation of ASD equipment**: Phileas® 250, Phileas® ONE in the rooms, Phileas® Genius in the BSC; **installation of indicators**
- 4.30pm: **HVAC stops**
- 4.40pm: "maintenance" type **BSC disinfection programme**: a long programme designed to disinfect the workspace as well as the plenum and filters, right up to the outlet filter (as opposed to a short "routine" programme, for disinfecting the workspace and below only).
- 5.40pm: **start of the equipment for the rooms, staff leave the area**. There is no need to tape the doors (unless a pressure difference with the adjacent area could cause air to enter or H2O2 to leak out, resulting in a loss of efficiency).
- 5 a.m.: **HVAC is restarted**
- 8am: **verification of residual H2O2 (<1ppm), staff entry, resumption of activity**

Cycle development

	CM4 Part 1		CM4 Part 2	
Protocols				
	Protocol 1	Protocol 2	Protocol 1	Protocol 2
Dose	“Safe” dose	“Safe” dose -30%	“Safe” dose	“Safe” dose -30%
Total diffusion time	1hr38	1hr18	1hr05	52 min
Contact time	Night	Night	Night	Night
Aeration time	3hr	3hr	3hr	3hr
Test conditions				
Temperature	23°C	23°C	22°C	22°C
Residual humidity	62-75%	62-72%	75%	68%
Consumables	Same batches O2SAFE 7.4®, chemical and biological indicators			
H2O2 level measured at 8pm	<1ppm	<1ppm	<1ppm	<1ppm
Results				
Diffusion compliance	Compliant	Compliant	Compliant	Compliant
% CI compliant	100%	100%	100%	100%
% BI compliant log10 6	100%	100%	100%	100%
Protocol validity	Validated	Validated	Validated	Validated
Conclusion				
Transition to PQ with Protool 2				

Table 3: protocols tested and results

Cycle development

Diffusions took place satisfactorily in the zones; as an example, image 1 shows a **diffusion report issued by the MyPhileas** application on diffusion by the Phileas® 250 during the 1er protocol of part 2 (room 91): 1,000mL theoretically to be diffused, 1,002.3mL diffused (the weight sensor integrated into the Phileas® 250 device makes it possible to accurately determine the quantity actually diffused). As the **zone** is **completely sealed**, the **humidity and temperature curves** are very **stable**, and the **recovery of HVAC** is clearly **visible** (graph 3).

Report

Phileas 250 S/N : 02011112

Version : 01.09/Build 2.0.37 Date : 20/06/2023

Report's time : 17h41 Operator : DEVEA

Zone Data

Room (5) Volume :

Temperature : 0.0°C

Hygrometry: 0.0%

Comments : CM4PARTIE2TEST1PIECE91

Chemical data

Chemical name : O2SAFE Batch number : FR2300002586

Expiry date : 22/04/2025

Program information

Phase 1 : Delay time is beginning: 16h59 (Duration: 0h11)

Phase 2 :

Diffusion starting : 17h10 - Diffusion end: 17h41 (Duration: 31 min)

Phase 3 : Contact time end: 17h41 (Duration: 0h00)

Calculated amount of liquid to spray : 1000.00 mL

Program conclusion

Actual duration of diffusion : 0h31

Real target amount of liquid sprayed : 1002.3 mL

Dissemination process completed :

Diffusion successful, quantity of disinfectant diffused compliant

Non compliance information :

-

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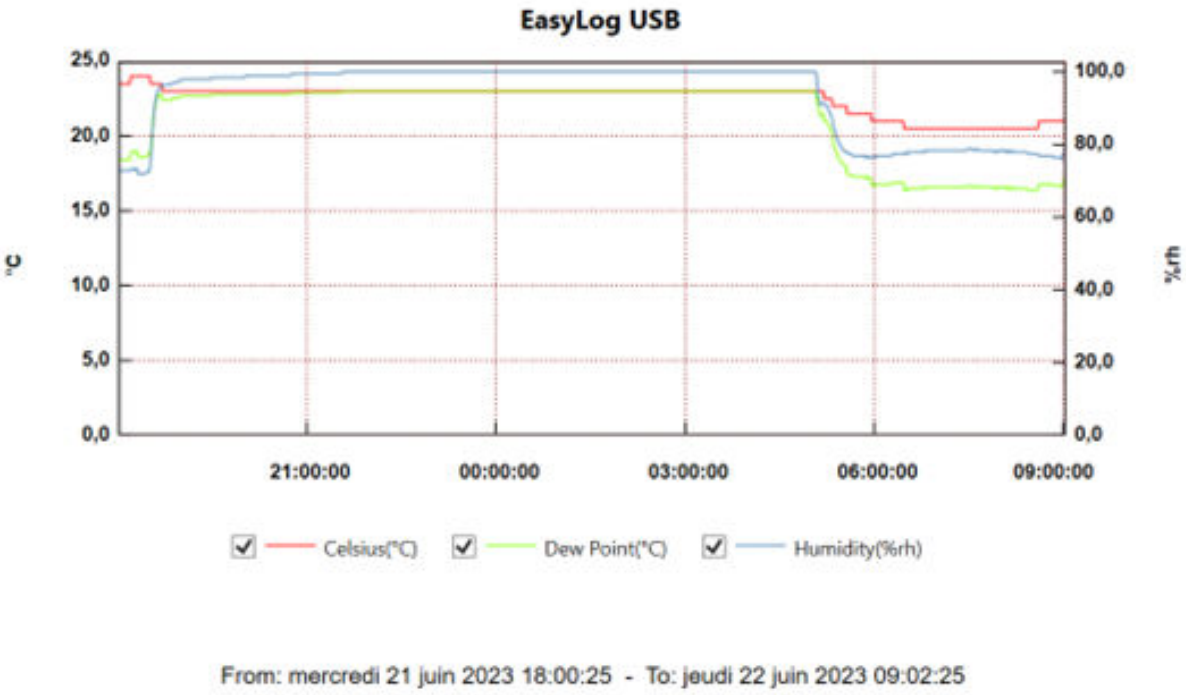
DEVEA Validation

Image 1: example of MyPhileas diffusion report

Cycle development



Graph 3: Temperature and humidity curve during ASD treatment

Image 2: Room 94 ready for ASD

Cycle development

Image 3: Room 160 (grey airlock) getting ready for ASD

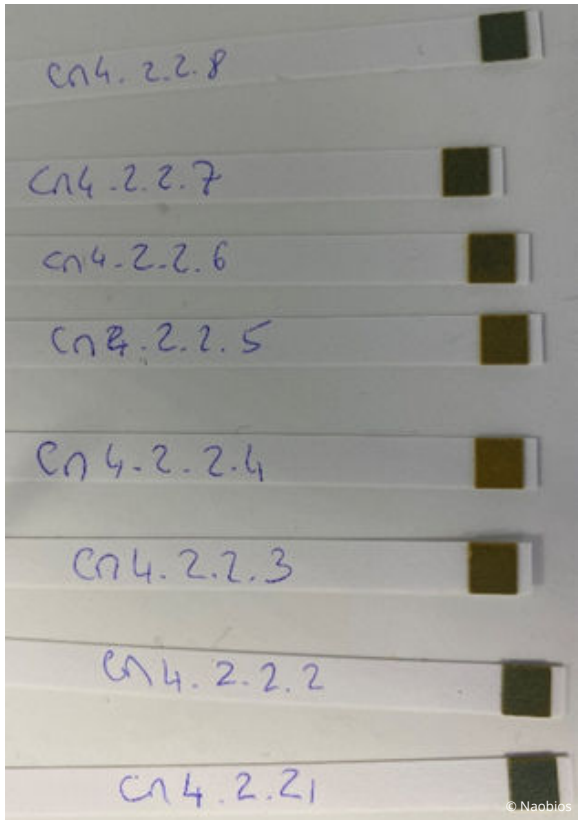


Image 4: coloured chemical indicators after ASD (CM4 / part 2 / protocol 2 / CI 1 to 8)

Cycle development

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All the **biological indicators** were **compliant** to expected logarithmic reduction (**log10 6**) in the rooms for both protocols. In the **BSC**, the **results** were also **excellent**: BIs up to 6-log10 in the workspace and below were treated as expected (also in the short cycle); the BSC plenum and the exhaust filter outlet were disinfected to log10 5, which exceeded the site's expectations.

Performance qualification

Protocol 2 was chosen for the PQ. Viral coupons were added to the *Geobacillus stearothermophilus* biological indicators, to **reflect the activity of these production zones**. A literature review led to the selection of the **Minute Virus of Mice strain** (ATCC reference VR-1346), one of the most resistant strains. For each new customer project, the biological material will be analysed to determine whether it is more or less resistant than the strain used for validation.

A total of **seven points** are chosen for the location of viral coupons, on the basis of a risk analysis (handling, leakage, risk of spillage): on work surfaces, in enclosures and inside BSCs (in the workspace). The viral coupons are prepared by the in-house Quality Control laboratory, with an initial population of 10e6 CFU, for a final target population of less than or equal to 10e2 CFU, i.e. a minimum reduction of 4 log10 of virus.

Performance qualification

	Partie 1			Partie 2			PSM		
Environmental conditions									
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
Initial temperature	21,2°C	19,0°C	18,6°C	19,1°C	20,5°C	19,4°C	23,1°C	21,9°C	18,1°C
Initial residual hygrometry	48,3 – 52,5%	37,5 – 44,3%	62,8%	43,3%	54,0%	58,5%	53,0%	59,5%	38,5%
Consumables	O2SAFE 7.4® batches: FR230169, FR237949, FR2300002586, FR2300010434, FR2300006440 Chemical indicators batches: 312327, 312302, 312245 Biological indicators batches: AZ-008, AH-216, AH-224, 23003, 23029, AP-048, AP-054 6-log10 pop. 1.3 à 2.4.10e6 CFU, d-value 1.3min								
Resuls									
Diffusion compliance	Compliant	Compliant	Compliant	Compliant	Compliant	Compliant	Compliant	Compliant	Compliant
% CI compliant	100%	100%	100%	100%	100%	100%	100%	100%	100%
% BI compliant log10 6	100%	100%	100%	100%	100%	100%	100% *	100% *	100% *
BI Triscale (site goal: 4 log10)	NA	NA	NA	NA	NA	NA	5 log10 reduction**	5 log10 reduction**	5 log10 reduction**
PQ test compliance	Compliant	Compliant	Compliant	Compliant	Compliant	Compliant	Compliant	Compliant	Compliant

Table 4: PQ tests and results on biological indicators (* in the class A enclosure, ** in the BSC technical plenum)

Performance qualification

	Part 1			Part 2			BSC		
Environmental conditions									
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
Initial temperature	21,0-23,0°C	20,4-21,0°C	18,6-21,2°C	20,8°C	21,0°C	21,1°C	23,1°C	21,9°C	20,0°C
Initial residual hygrometry	60-64,5%	52,8-64,0%	48,3-52,5-62,8%	48,8 %	64,5%	87,5%	53,0%	59,5%	77,0%
Consumables	See table 4								
Results									
Diffusion compliance	Compliant	Compliant	Compliant	Compliant	Compliant	Compliant	Compliant	Compliant	Compliant
% CI Compliant	100%	100%	100%	100%	100%	100%	100%	100%	100%
Minimum viral reduction: goal 4 log10 (TCID50/mL)	> 5,5 log10	> 5,9 log10	> 4,3 log10	> 5,9 log10	> 6,2 log10	> 6,1 log10	> 5,5 log10	> 5,9 log10	> 5,5 log10
QP test compliance	Compliant	Compliant	Compliant	Compliant	Compliant	Compliant	Compliant	Compliant	Compliant

Table 5: PQ tests and virus coupon results

Conclusion

In less than six months, the cycle development and the PQ of this BSL-3 biotechnology laboratory have enabled an ASD protocol to be validated with **6 log10 disinfection (BI) and 4 log10 (MVM viral coupons)**, for the area and the associated BSC (6-log10 in the class A work chamber, 5-log10 achieved in the technical plenum). The teams in this production area have gained in particular:

- safety for operators,
- ease of implementation and
- savings in terms of number of devices and energy efficiency (only three devices per zone, cold diffusion).

The ASD is robust and repeatable, and now includes the BSC, with excellent results for the plenum and filters, and optimal installation.

Qualification of the other three production zones has been performed at this site since then.

Thank you!

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