Technical Data Monograph

VIRASURE™ Air Decontamination System for Steam Sterilization and Effluent Decontamination

STERIS
Life Sciences

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1. Abstract

Steam sterilization of bio-hazardous loads in autoclaves, and storage and decontamination tanks of effluent decontamination systems, requires uncompromised safety and process integrity. The risks arise from the potential contamination of the surrounding environment in the sterilization or effluent decontamination process. Applications associated with these risks are categorized in one of four bio-safety levels, with three and four being the most critical in terms of process integrity.

To mitigate this risk conventional methods have utilized the following:

- Electrically heated multiple heating elements or directly electrically heated tube "incinerator" type structure with flow baffles
- Single or dual vent filter arrangements classified as HEPA or membrane filters that commonly have pore size of 0.22 µm to prevent micro-organisms from spreading out through vent connection during process air exchange.

The VIRASURE air decontamination system is different due to its combined forced hot surface contact, coupled with a heated 0.1 µm straining element, all contained in a controlled fail-safe process environment. This improves the safety and redundancy of sterilizer decontamination cycle or tank containment integrity.

2. VIRASURE applications

Applications typically requiring such safety requirements for their autoclave processing and effluent decontamination are found in, but not limited to: pharmaceutical manufacturing, research utilizing bio-hazardous micro-organisms, lab animal research facilities with high containment requirements, hospital infection control, and pathology departments.

Autoclave Sterilization

Autoclave processing in high containment applications, such as those previously mentioned, utilizes a decontamination cycle. To provide assurance that any living bio-hazardous micro-organism contained in the load at the start of the sterilization cycle is not inadvertently pulled out of the chamber into the drain during the first vacuum pulse of the sterilization cycle, the typical removal of air through the drain while steam is injected into the chamber is reversed. Steam is injected through the drain and air is removed through the top of the sterilizer through a HEPA or membrane filter with a pore size of 0.22 µm filter. This effectively traps any pathogens between the 0.22 µm filter and the lethality of the steam ensuring the containment of any pathogens. In some cases 0.22 µm filters are installed in series for added protection.

In the decontamination autoclave, VIRASURE replaces the 0.22 µm filter(s) and increases the safety and redundancy, by combining *heat* and *small pore size straining*. As the diagram in Figure 2 depicts, the VIRASURE is comprised of two heated elements. The first element is a tightly arranged system of heated tubes and baffles, through which air is forced. The second element is not only heated, but is also a 0.1 µm strainer. Adding to the safety of this system is permanent nature of the installation, so

cartridge replacement or integrity testing related to cartridge change is not required for the VIRASURE heated strainer element. The entire assembly is a welded structure; this also increases safety by minimizing any possible leaks. The process is as follows:

Process Sequence

1. VIRASURE heating element is brought to pre-set temperature (200 °C to 400 °C) and vacuum pump is engaged and outside air is brought through air inlet filter.
2. Once preset temperature is reached, the air filter is closed and the air in the chamber is routed through VIRASURE.
3. Steam is routed through the drain into the chamber (the piping and flow of steam is opposite from that of a standard sterilization cycle.) Vacuum pulses and steam injection are continued until the temperature and pressure in the chamber have reached their preset parameters. Throughout this process, the VIRASURE Air Decontamination System is continuing to sterilize air and steam being pulled out of the chamber prior to reaching vacuum pump.
4. Once temperature and pressure are met, conditions are held for the length of the set exposure / sterilization phase.
5. Post-conditioning is carried out as in other standard sterilization cycles.

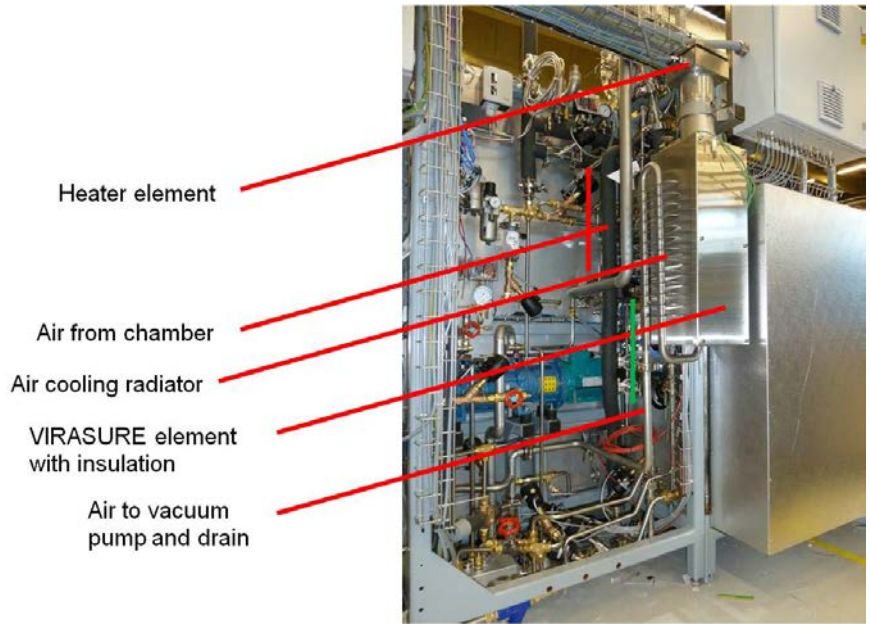


Figure 1

Continuous Effluent Decontamination

In CED (Continuous Effluent Decontamination) applications the VIRASURE element is installed on top of the effluent collection buffer tank to protect the surroundings from potentially contaminated air exiting the tank as a result of effluent water discharged to the tank. In this way the collecting tank is maintained in atmospheric pressure condition to allow balancing of effluent flows collecting to the tank from different equipment and drainage points. Physical installation can be vertical or horizontal, depending on available room height.

In this application the equipment setup is similar to one in sterilizers, with the exception that in the normal operation mode the VIRASURE element is continuously heated. Since contaminated air

leakage from the connection to the contaminated tank interior is a risk, the fully welded structure of the VIRASURE becomes an especially important feature of the installation in that it provides additional safety and redundancy in BSL-4 applications that typically require dual filter elements setup or incinerators on vent lines.

Since service may be required, the VIRASURE element is equipped with a port through which steam can be directly injected, this process section can be isolated and steam sterilized by direct steam injection through the element and its piping route to ensure that the adjoining sections and components are also safe to operate on.

The following schematic shows the air flow through the VIRASURE element in both sterilizer and effluent collecting tank applications.

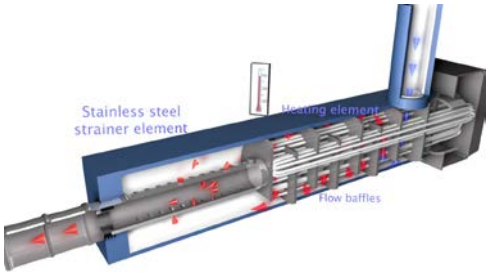


Figure 2

Image 1: VIRASURE element air flow schematic

Figure 3

Image 3: VIRASURE element placement on top of effluent collecting tank

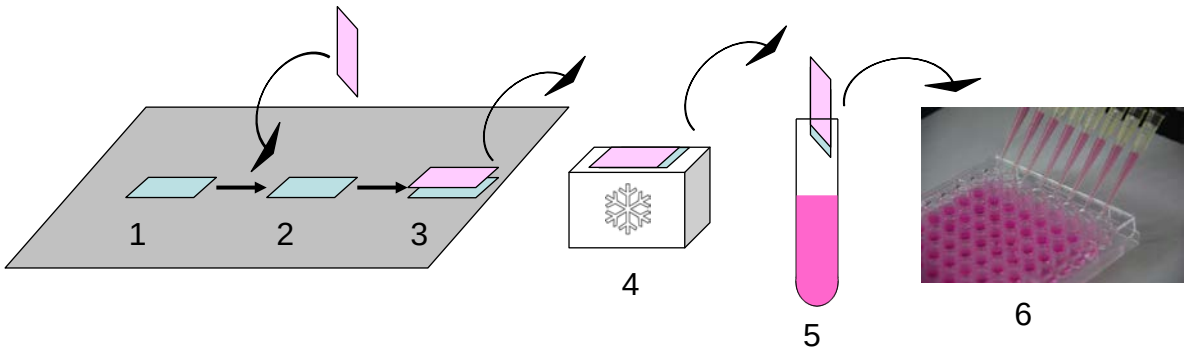
3. VIRASURE Efficacy Testing with *Parvovirus* & *Bacillus Atropheus*

Efficacy of forced hot contact was tested by STERIS CORPORATION in laboratory conditions[1] in different temperatures to establish a time/temperature relation in kill efficacy for *Porcine Parvovirus* and *Bacillus Atropheus.*

For *Bacillus Atropheus* spores, 20 µL of a 1.6 x 10^8 / mL spore suspension in water were deposited onto clean sterile stainless steel coupons (dimensions: 1x3cm) and allowed to dry under a laminar flow hood for 1 hour.

For *Porcine Parvovirus*, 25 µL of a 5.0 x 10^8 TCID50 / mL virus suspension in OptiMEM + 10% FBS was deposited onto clean sterile stainless steel coupons (dimensions: 1 x 3 cm) and allowed to dry under a laminar flow hood for 1 hour.

Dry heat exposures were then performed by putting contaminated coupons in direct contact with support sterile coupons placed on a heating plate and pre-heated at the designated test temperature for 30 min (as shown below). This procedure was controlled to be appropriate by measuring temperature at the surface of pre-heated coupons using a temperature probe. Controls were performed with spores or viruses dried onto coupons and incubated at room temperature for approximately 30 min.



1: A clean, sterile stainless steel coupon (1 x 3 cm) was placed on a heating plate and pre-heated at the specified test temperature for 30 min.

2: Viruses or spore contaminated coupons were placed in direct contact with the pre-heated coupons.

3: Coupons were left in contact with the heating plate for 1 to 10 min.

4: Coupons placed on a pre-cooled (+4 °C) metal block for 1 min.

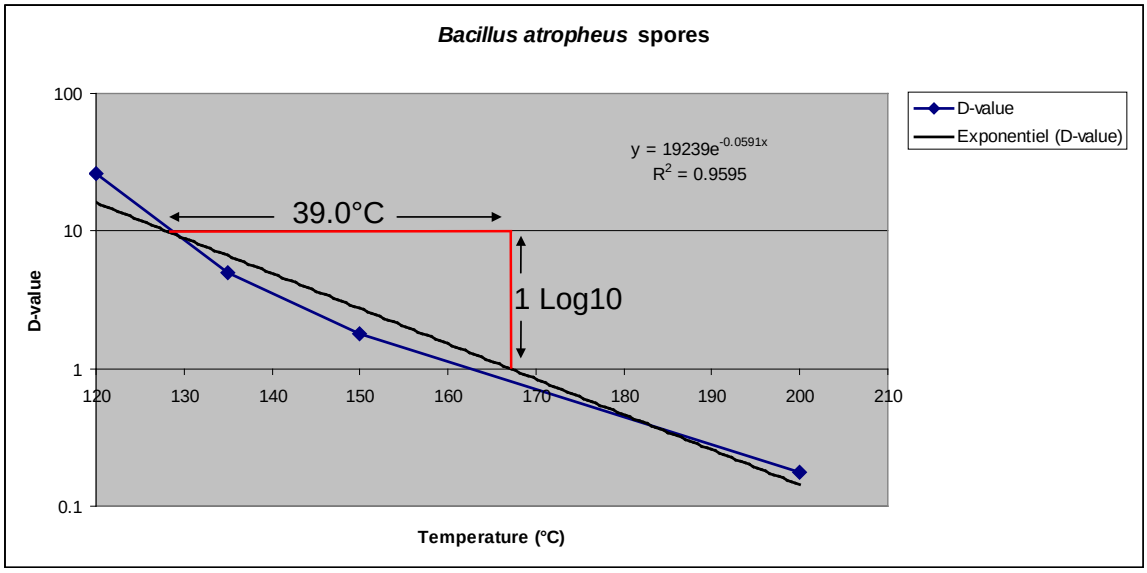
5: Coupons were transferred into 1 mL cell culture medium (for virus) or tryptone broth (for spores) and submitted to vigorous vortex for 1 min.

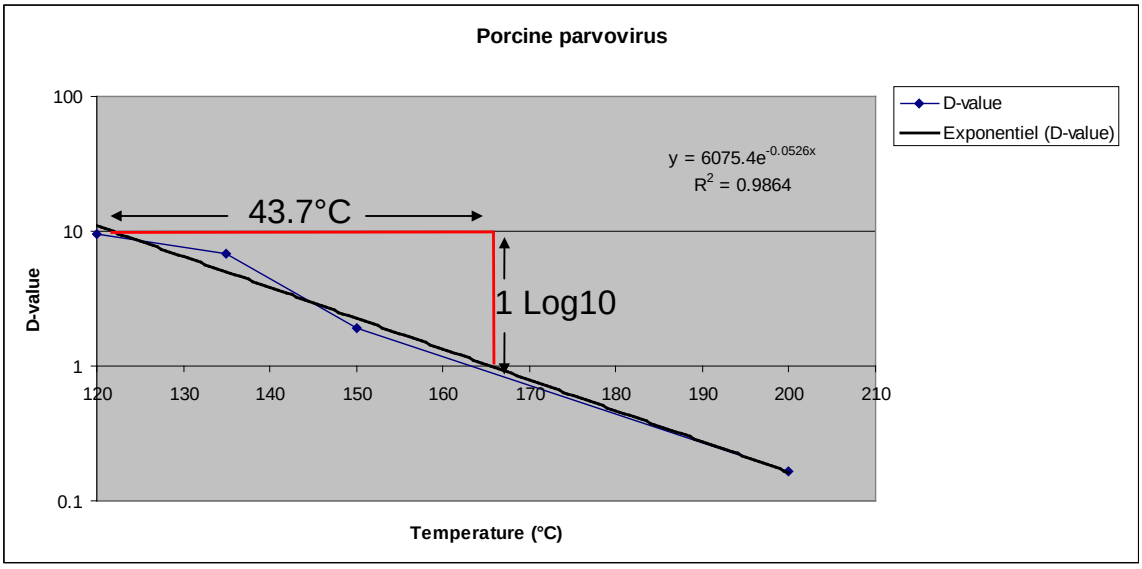
6: Recovered suspension was then serial-diluted on susceptible cells (virus) or tryptone broth.

For virus testing, infected cells were infected for 7 days at 37 °C + 5% CO_2 to observe cytopathic effect. Spores were incubated in tryptone broth for 3 days at 37 °C to allow for germination and growth. They were then observed for media colour change and turbidity. In both cases, residual titres were calculated using the modified Spearman-Kärber method (see Annex 1). All tests were performed in duplicate on two occasions.

The test results are summarized in the following graphs (figures 1 & 2) representing regression of D-values on a logarithmic scale according to temperatures.

Figures 1& 2: Regression curves calculated for D-values against temperatures on a semi-logarithmic scale.





Conclusions

This data demonstrates that *Bacillus Atropheus* NCIMB 8649 spores and porcine parvovirus ATCC VR-742 present very similar kinetics of inactivation when exposed to dry heat under the conditions tested.

Exposure to higher temperatures for sterilization applications should provide the following estimated D-values (Table 1).

Table 1: D-values calculated for spores and viruses

Temperature	D-values (seconds)	
	Spores	Virus
200 °C	8.49	9.84
250 °C	0.44	0.71
300 °C	0.023	0.051
350 °C	0.0012	0.0037
400 °C	0.000062	0.00027

[1] *Eterpi et al: Dry Heat Sterilization Efficacy against Porcine Parvovirus and Bacillus Atropheus Spores (STERIS CORPORATION 2011)*

4. VIRASURE Flow Rate Test for Process Integrity

Since the integrity of the VIRASURE heated strainer is of critical importance, the following testing cycle has been developed and is installed into the autoclave or CED controller.

Each sterilizer decontamination process has a pre-cycle test that ensures the operating condition of the VIRASURE heated strainer element. The test is a pre-cycle step that measures the time elapsed from low vacuum pressure level created in the chamber by vacuum pump (typically 1.5 psia) to atmospheric pressure when the air flow is directed through the VIRASURE system. The record of this cycle is captured and printed.

For example, in a 669-size sterilizer chamber, a typical pressure equalization time would be 22to 23 seconds. Equalization time varies due to different chamber sizes, and is therefore defined for each unit individually. Test is performed 3 consecutive times to determine mean equalization time.

This test works for two purposes:

1) Ensuring there are no leaks in the VIRASURE strainer or system
2) Ensuring the strainer element is not clogged over time

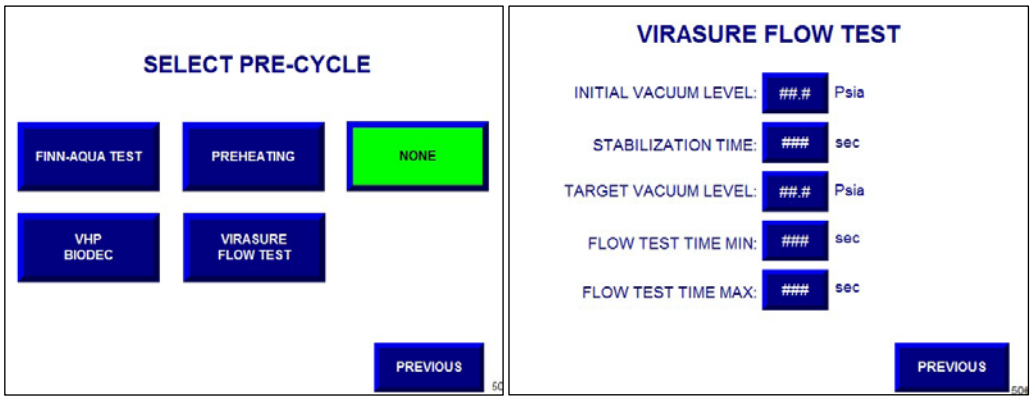
For VIRASURE system this test is conducted during the factory acceptance test (FAT) to record the reference time while the system is new. After start-up of sterilizer, the user can periodically re-test to ensure the condition of the system.

Therefore a minimum and maximum time range is set for the flow rate test. Using the above mentioned 669-size sterilizer chamber as an example, the acceptable range would be 20 to 100 seconds, when the recorded equalization time at FAT is approximately 22 to 23 seconds.

Test result of < 20 seconds would indicate a possible leak.

Test result exceeding 100 seconds suggests the element is clogged and needs to be cleaned / inspected.

An out-of-range measurement result leads to failed test.



5. VIRASURE Process Redundancy for Safety

Process redundancy is required in decontamination applications. From a risk management perspective, more than one back-up function is required to ensure safe operation in case of component, program or utility failure no matter of the likelihood In the case a process cycle is aborted due to a critical alarm, the cycle is aborted into a fully contained fail-safe mode.

The result is that the VIRASURE Air Decontamination System protects the surrounding environment from any contamination, and the process is continuously monitored through duplicated temperature measurements.

There are four different independent control measures for the operation of VIRASURE:

1. Primary process temperature sensor (monitored by PLC), also operates as over-temperature protection for the heating element. Failure to reach process temperature or critical sensor failure will alarm and abort the cycle.
2. Secondary process temperature sensor (monitored by PLC), also operates as over-temperature protection for the heating element. Failure to reach process temperature or critical sensor failure will alarm and abort the cycle.
3. Redundant process temperature sensor (monitored by hard-wired controller outside of PLC). This will lead to failure to reach process temperature or critical sensor failure will alarm and abort the cycle.
4. Contactor/relay for each heating element phase (3~) – measuring sufficient electrical current over each phase. PLC aborts the process if any of the heater element coils fail.

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